ARTICLE - Acute Myeloid Leukemia

Bleximenib, the novel menin-KMT2A inhibitor JNJ-75276617, impairs long-term proliferation and immune evasion in acute myeloid leukemia

Shanna M. Hogeling,[1] Duy Minh Lê,[1] Nikita La Rose,[1] Min Chul Kwon,[2] Albertus T.J. Wierenga,[1] Fiona A.J. van den Heuvel,[1] Vincent van den Boom,[1] Anna Kuchnio,[2] 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 and [2]Discovery Oncology, Janssen R&D, Beerse, Belgium.

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

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Supplemental Figures and Methods

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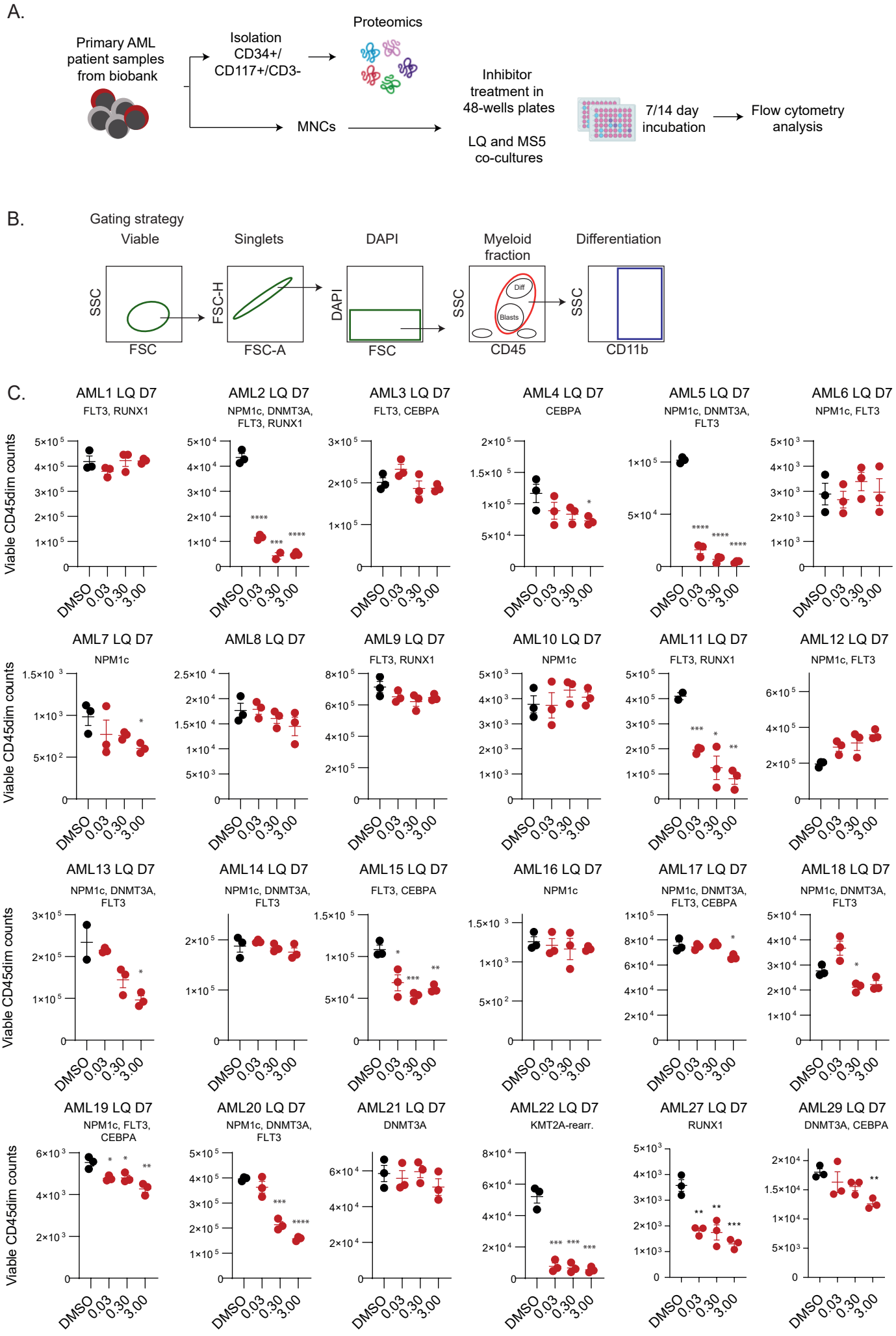
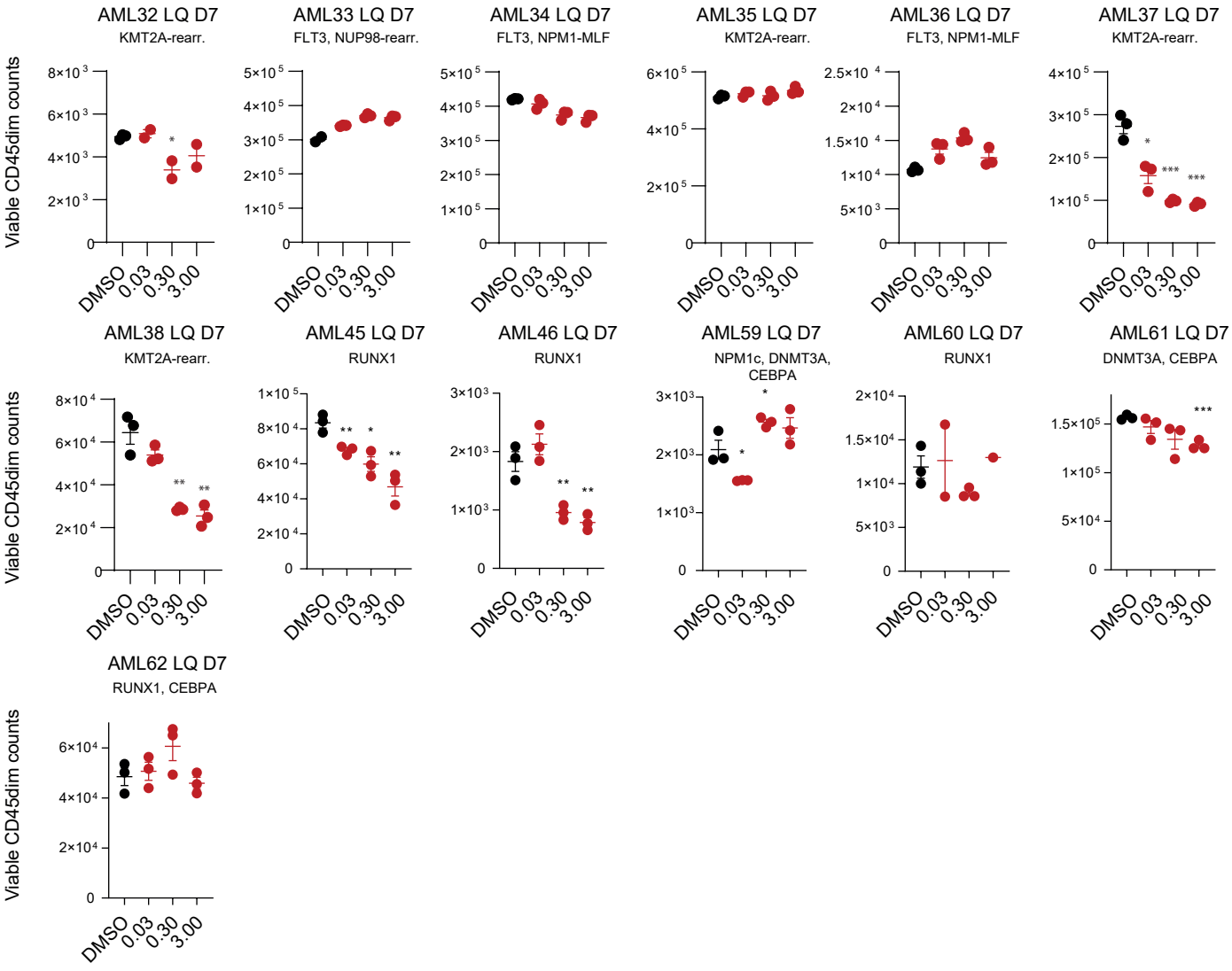
Shanna M. Hogeling[1], Duy Minh Le[1], Nikita La Rose[1], Min Chul Kwon[2], Albertus T.J. Wierenga[1], Fiona A.J. van den Heuvel[1], Vincent van den Boom[1], Anna Kuchnio[2], Ulrike Philippar[2], Gerwin Huls[1] and Jan Jacob Schuringa[1*]

[1]Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [2]Discovery Oncology, Janssen R&D, Beerse, BE

Supplemental figure 1



Supplemental figure 1 - continued



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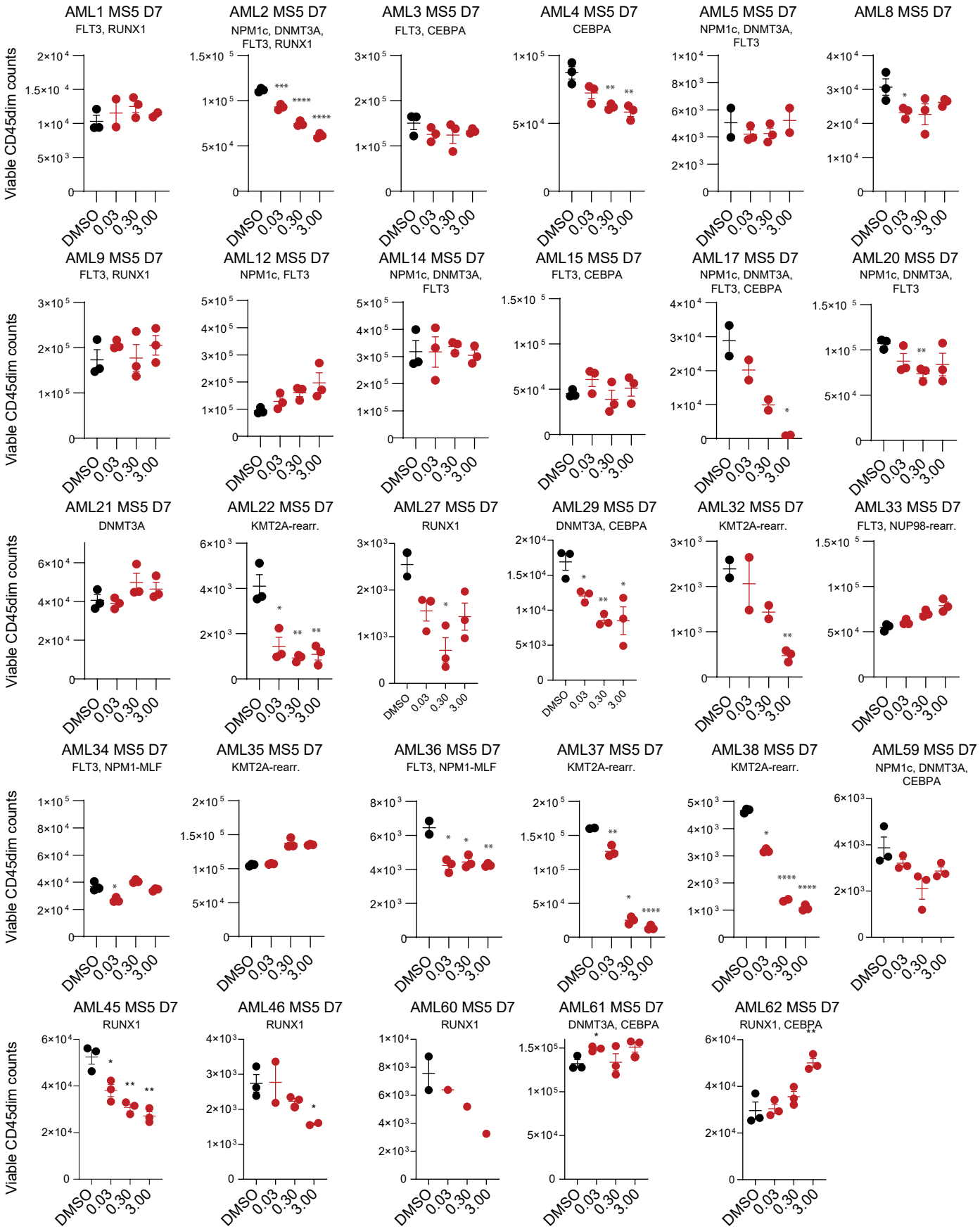

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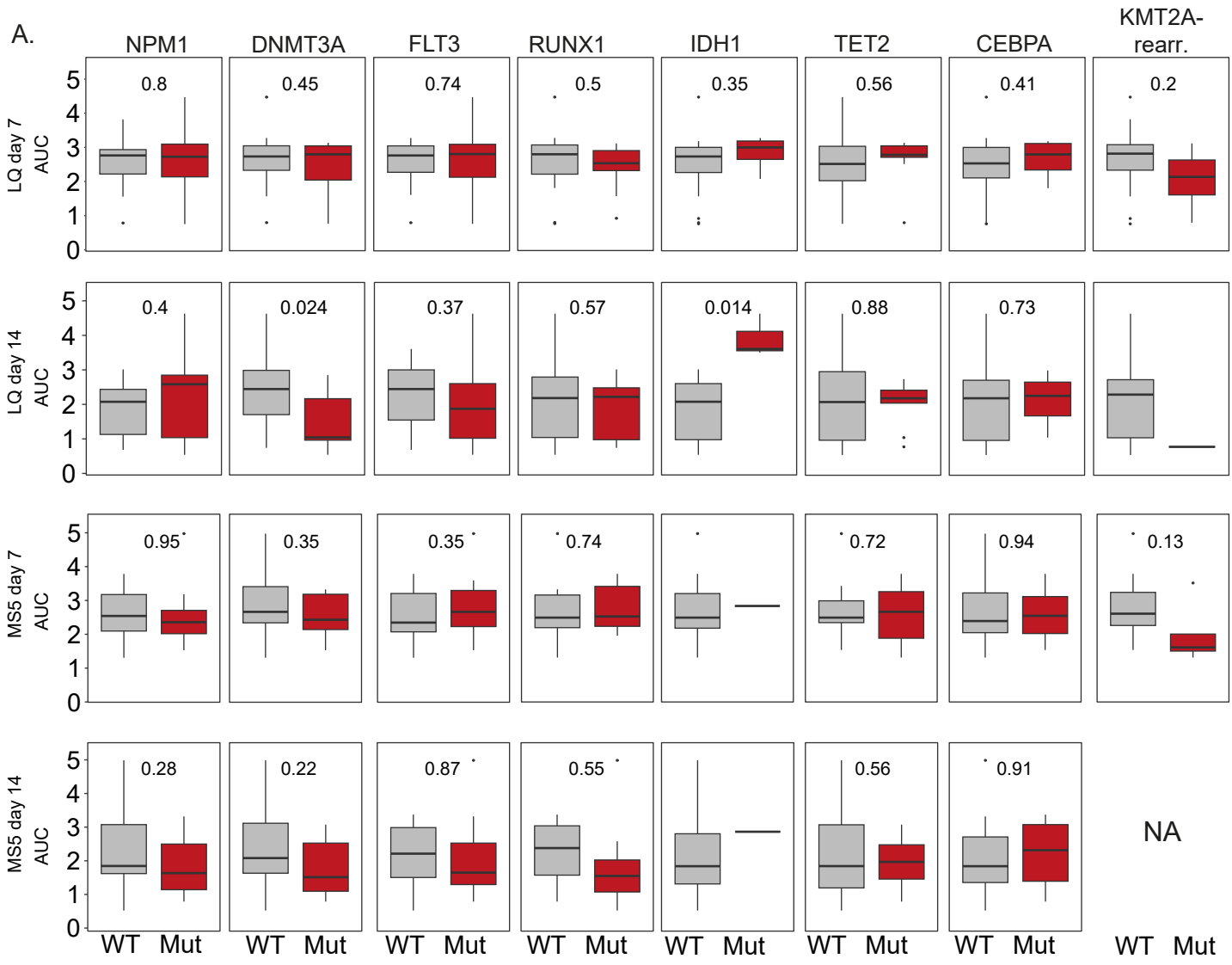
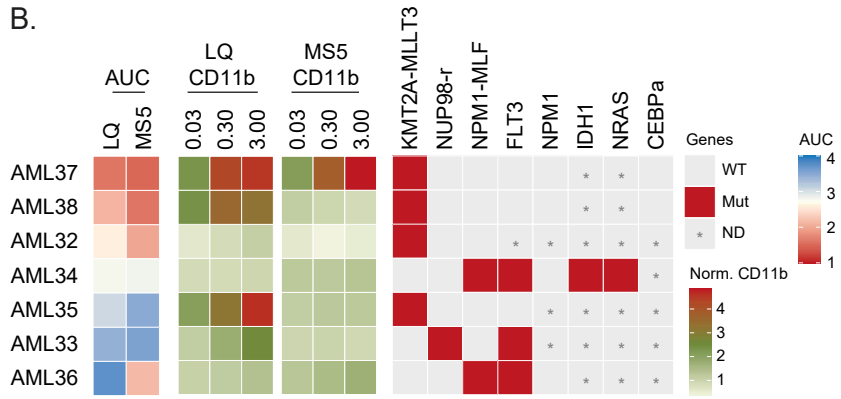
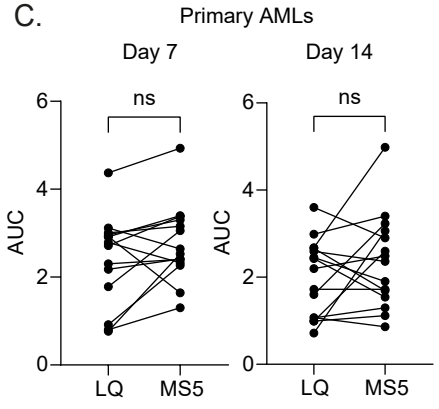
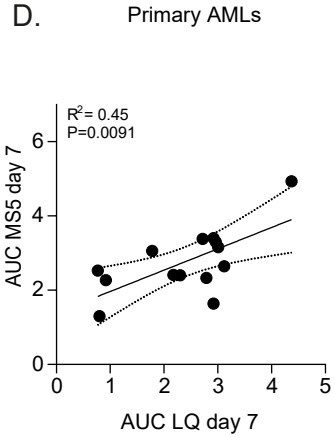
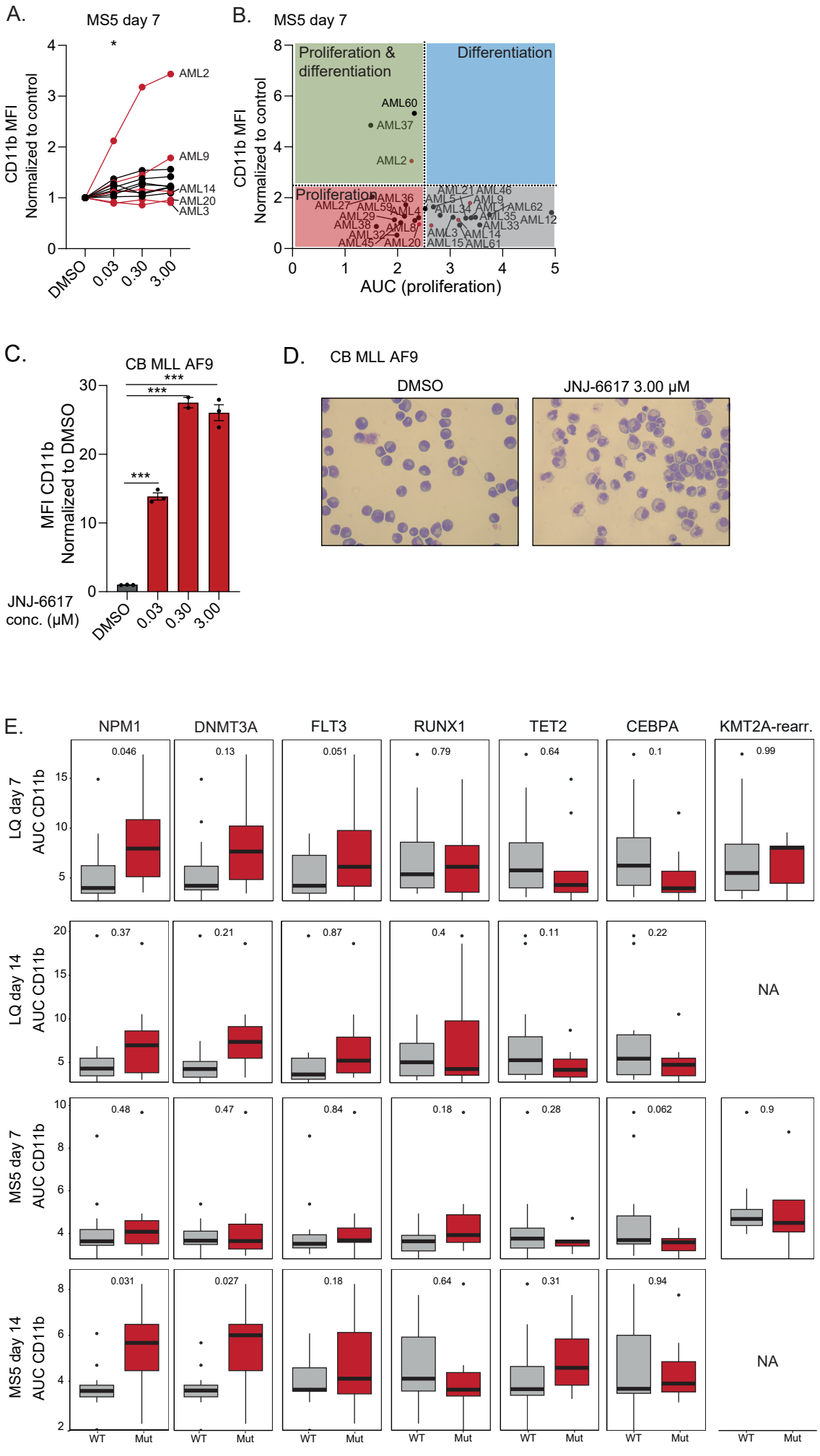




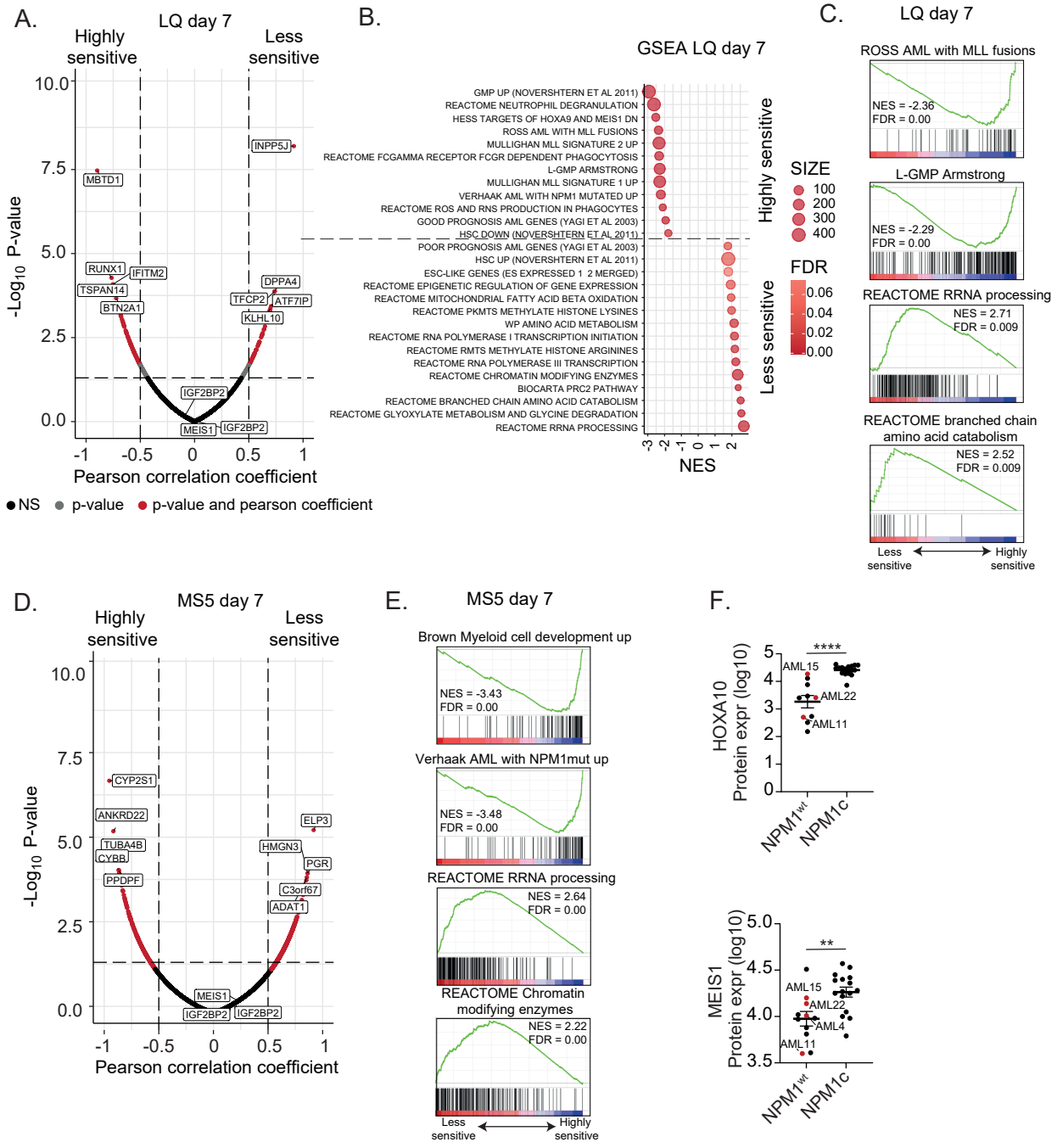




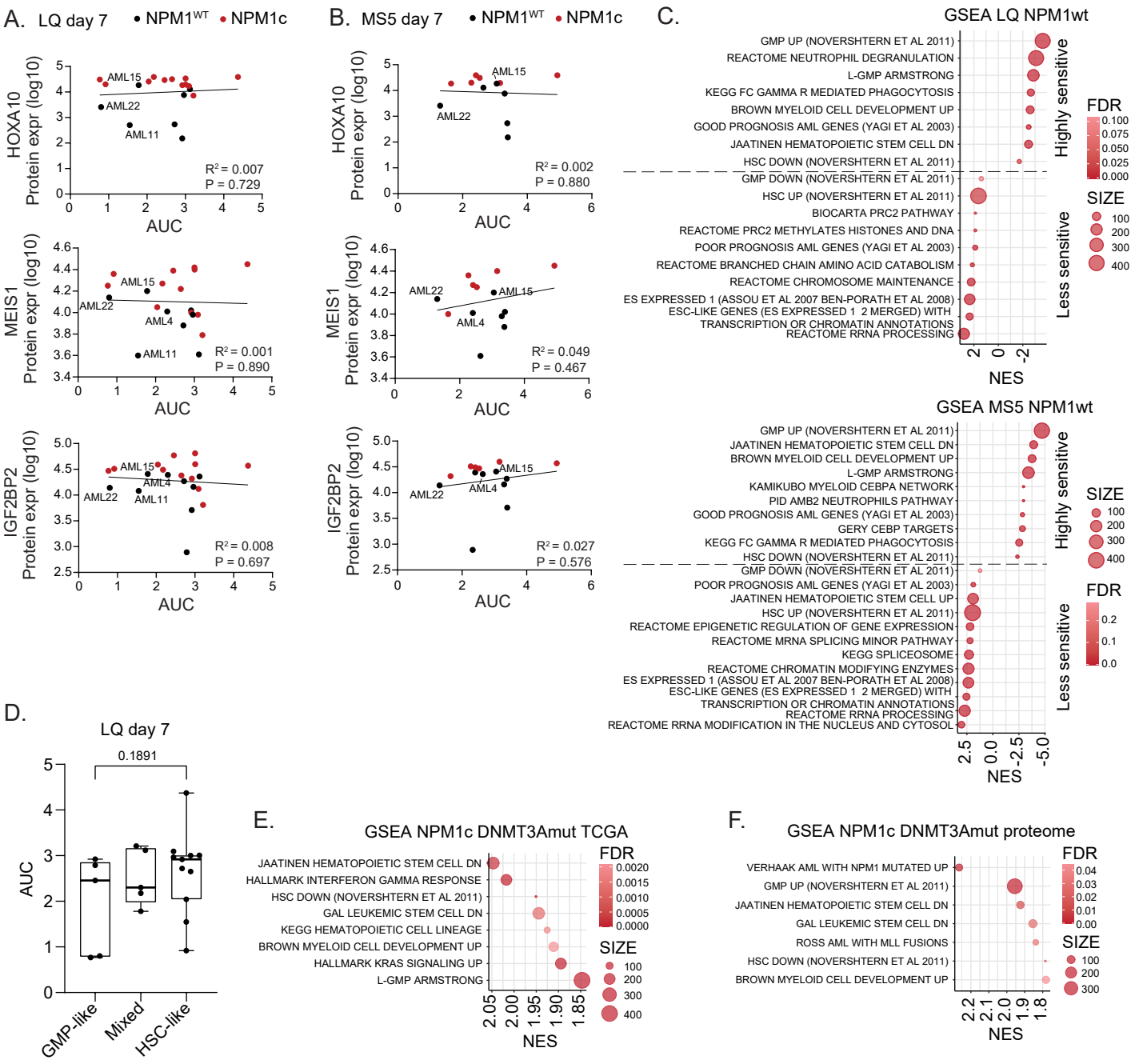
Supplemental figure 4



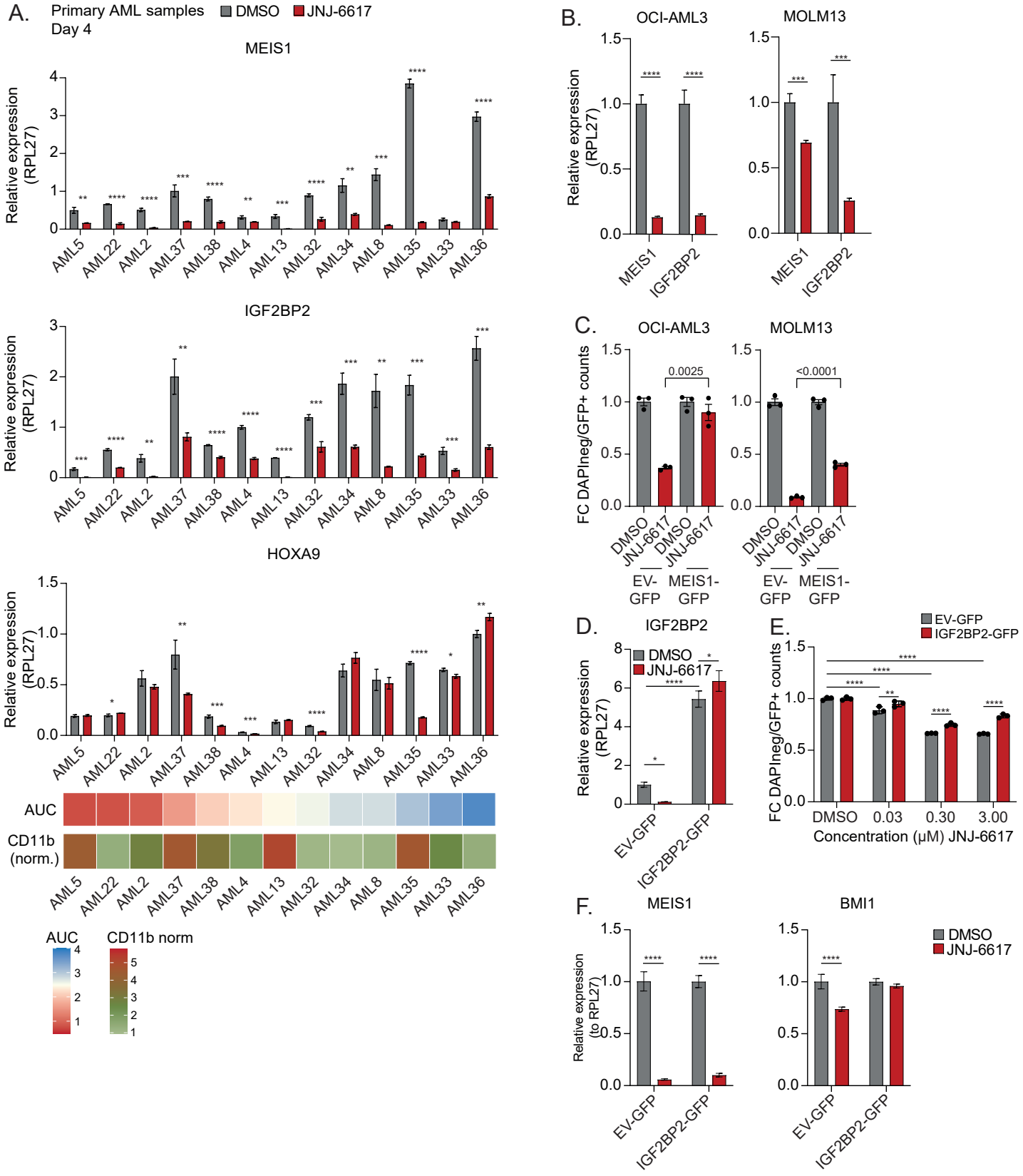
Supplemental figure 5



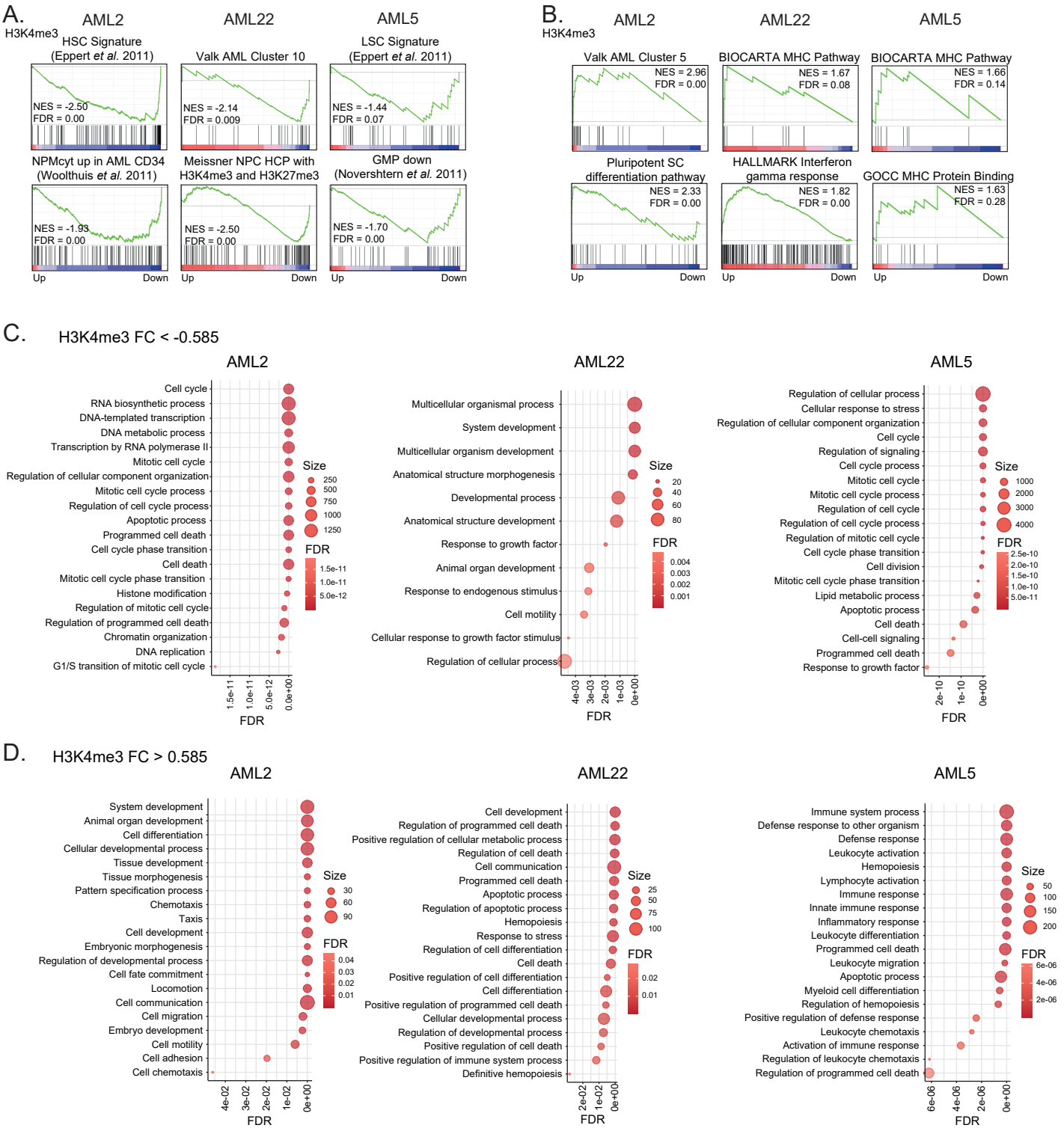
Supplemental figure 6



Supplemental figure 7

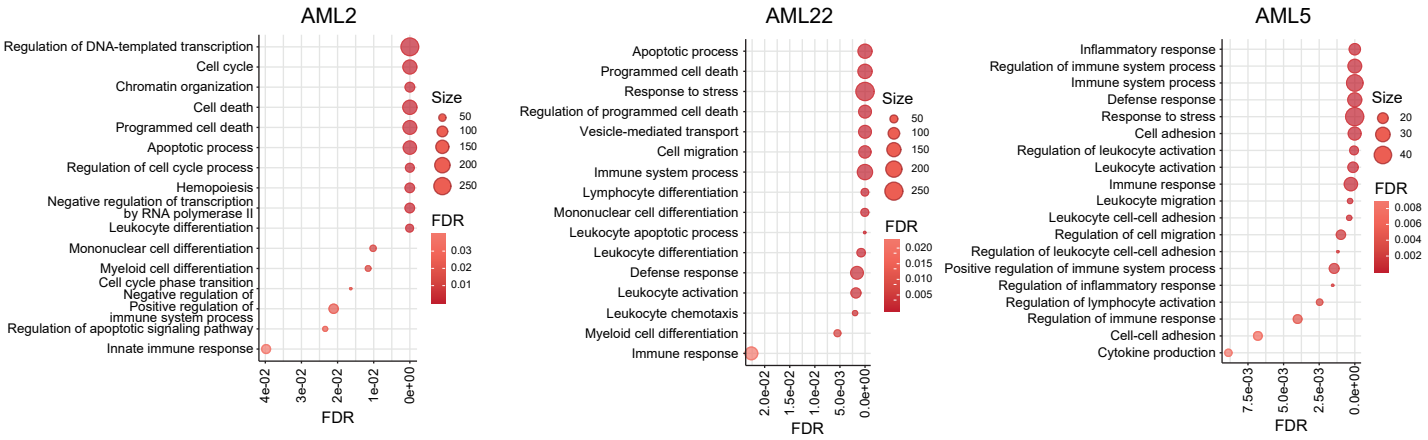


Supplemental figure 8

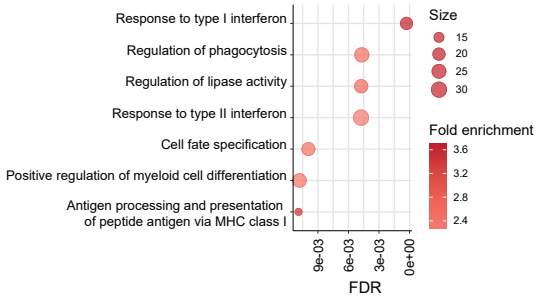


Supplemental figure 9

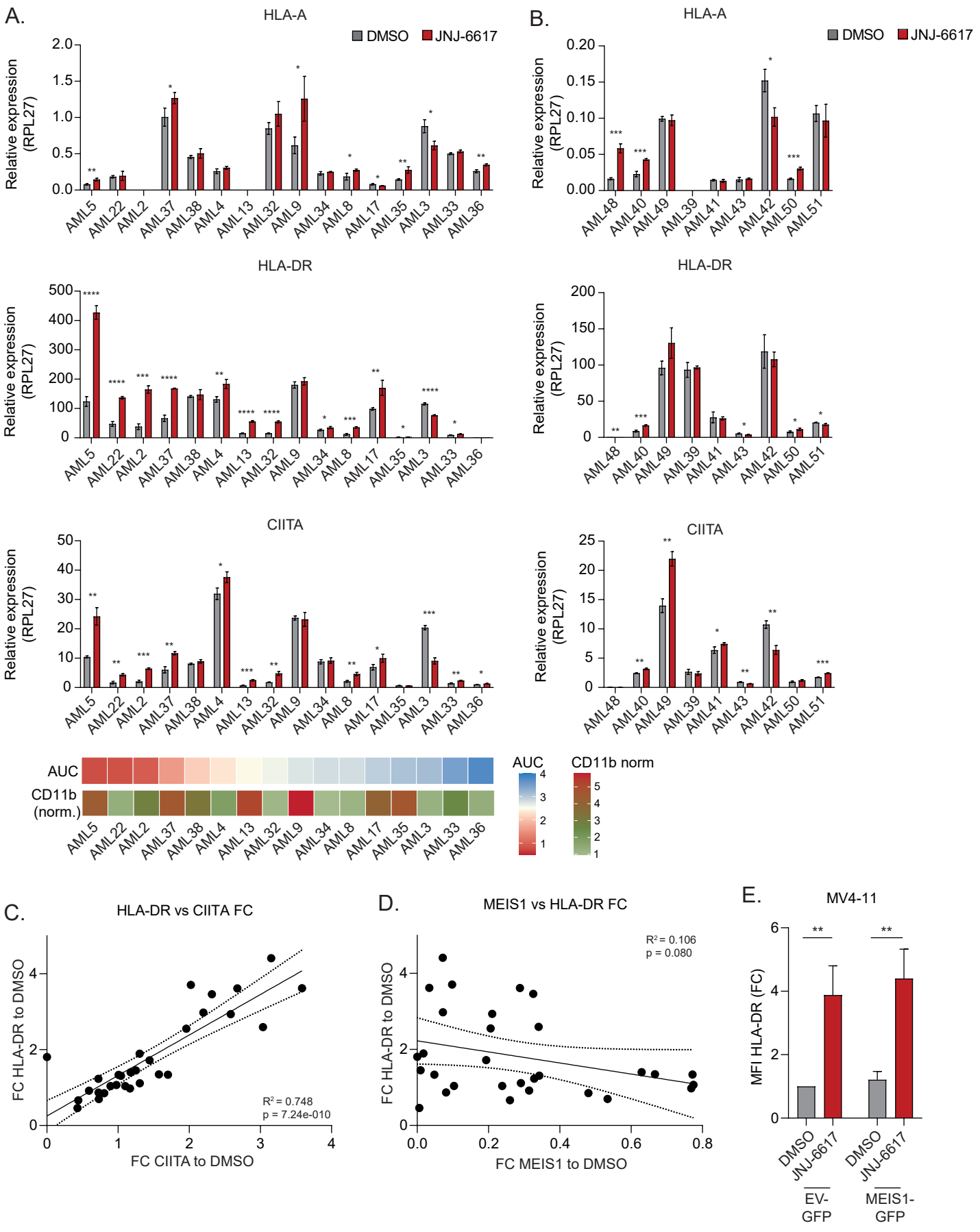
A. H3K27ac FC > 0.585



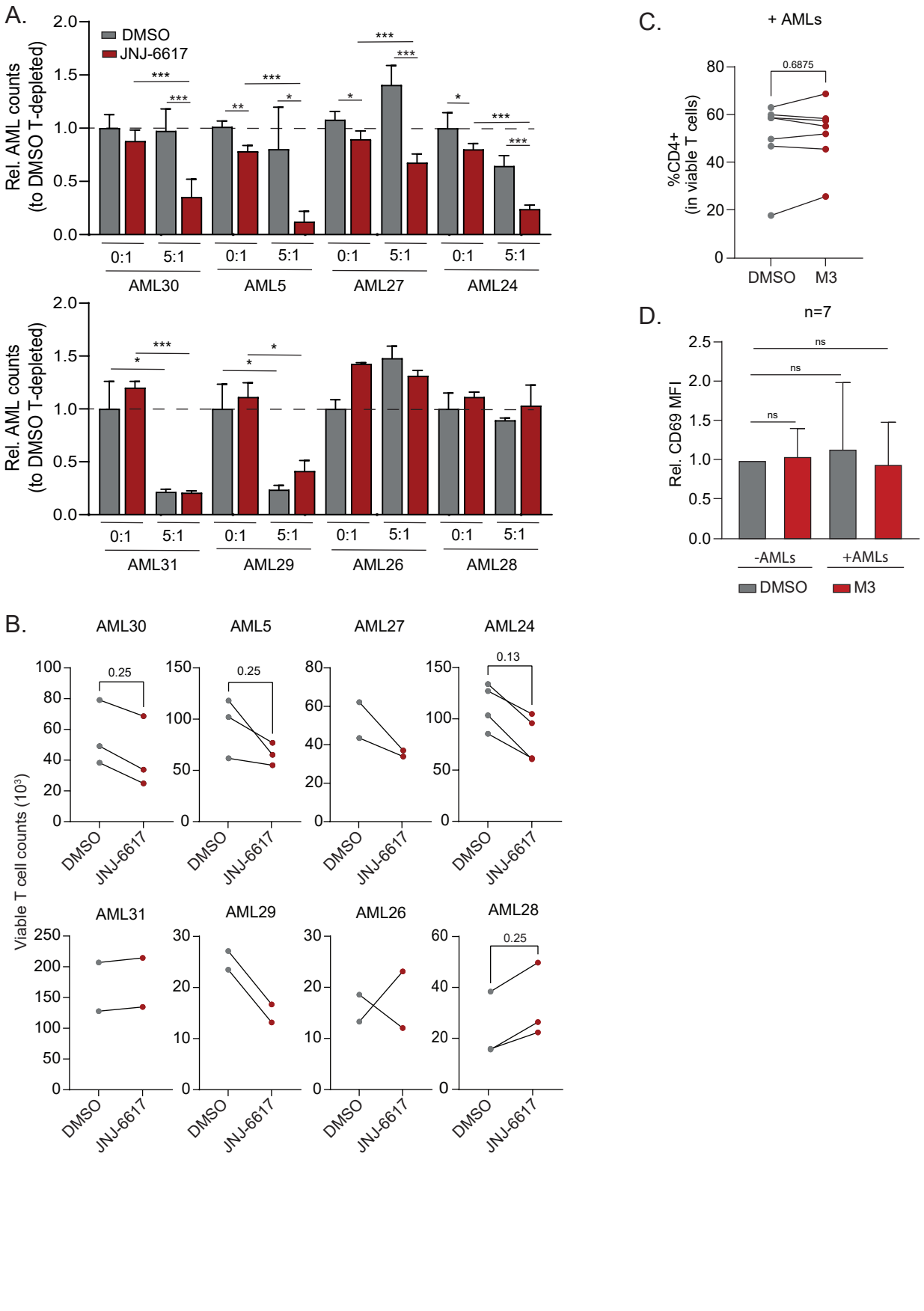
B. GSEA analysis H3K4me3 OCI-AML3 FC > 0.585



Supplemental figure 10



Supplemental figure 11



Supplemental table 1. AML studies used in the study

		Mutations																			AUC							FC compared to DMSO T-depleted		FC compared to DMSO T-depleted		FC compared to DMSO (MFI)			FC compared to DMSO (mRNA expression)						
AML	material	FLT3	NPM	DNMT3A	IDH1/2	TET2	ASXL1	RAS	WT1	RUNX1	CKIT	Splicing SF3B1, SRSF2	CEBPA	EZH2	U2AF1	BCOR/BCORL1	ANKRD26	MYC	GATA2	karyotype	LQ 7	LQ 14	MSS 7	MSS 14	ChIPseq	LFQ proteome	T-cell killing assay	FC T-cell killing 0:1	FC T-cell killing 5:1	FC T-cell killing (depleted)	FC T-cell killing (enriched)	MHC class I (HLA-A/B/C)	MHC class II (HLA-DR/DQ/DP)	HLA-DR	MEIS1	IGF2BP2	HOXA9	HLA-A	HLA-DR	CIITA
AML1	PB	0.1 (c.17	wt	wt	wt	wt	wt	wt	wt	0.44 (c.72	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK	2.9	2.7	3.4	5.0		yes															
AML2	PB	ITD	0.5	0.5	wt	wt	wt	wt	wt	0.55 (c.72	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK	0.9	1.0	2.3	1.1	yes	yes								1.487179	0.07	0.03	0.86		4.41	3.15
AML3	BM	0.35 (c.1	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	0.5 (c.89	wt	wt	wt	wt	wt	wt	wt	NK	3.1	1.7	2.6	1.7		yes										0.26	0.30		0.70	0.66	0.44
AML4	PB	wt	wt	wt	wt	wt	wt	wt	wt	wt	0.23 (c.2	wt	0.05 (c.5	wt	wt	wt	wt	wt	wt	wt	inv16	2.3	3.0	2.4	3.4		yes								1.5822	0.63	0.38	0.55	1.19	1.40	1.18
AML5	BM	0.3 (c.17	0.36 (c.8	0.48 (c.2	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK	0.8	0.6	2.5		yes	yes	allogenic	0.78	0.12							0.33	0.06	1.02	1.83	3.46	2.32
AML6	PB	0.51 (c.1	0.43 (c.8	wt	0.34 (IDH	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK	3.1	4.6				yes															
AML7	BM	wt	0.42 (c.8	wt	0.34 (IDH	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	nd	2.0	3.6		2.9		yes															
AML8	BM	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt	NK	2.8	2.5	2.3	1.9		yes										0.07	0.13	0.95	1.52	2.98	2.20
AML9	PB	0.12 (c.2	wt	wt	wt	0.32 (c.3	0.31 (c.1	wt	wt	0.48 (c.4	wt	wt	wt	0.99 (c.2	wt	wt	wt	wt	wt	wt	nd	2.7	2.4	3.4	1.5		yes										0.78	0.33		2.02	1.07	0.98
AML10	PB	wt	0.38 (c.8	wt	0.35 (IDH	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	nd	3.2	3.5				yes															
AML11	PB	ITD	wt	wt	wt	wt	0.5 (c.20	wt	wt	0.98 (c.4	wt	wt	wt	0.98 (c.2	wt	wt	wt	wt	wt	wt	nd	1.5	0.8				yes															
AML12	PB	ITD	0.38 (c.8	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	nd	4.4	10.0	4.9	3.3		yes															
AML13	PB	0.27	0.42 (c.8	0.48 (c.2	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	nd	2.5	1.0		2.6		yes								1.154255	0.03	0.06	1.14	4.60	3.62	3.59
AML14	BM	ITD	0.37 (c.8	0.4 (c.26	wt	0.3 (c.51	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK 46,XY[20]	3.0	2.2	3.2	2.5		yes															
AML15	BM	ITD	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	46,XX,t(11;17)(p15;q21)[10]	1.8	1.6	3.1	3.2		yes															
AML16	BM	wt	0.33	wt	wt	0.42	wt	wt	0.05	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	nd	2.7	2.7																			
AML17	PB	0.29 (c.1	0.38 (c.8	0.42 (c.2	wt	0.51 (c.5	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	46,XX	2.9	1.1	1.6	0.9		yes								0.19			0.75	1.72	1.44
AML18	PB	0.15 (c.2	0.36 (c.8	0.43 (c.2	wt	wt	wt	wt	0.44 (c.1	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	46,XX	3.0	2.7		1.7		yes															
AML19	PB	0.2 (c.17	0.39 (c.8	wt	wt	0.51 (c.3	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	46,XX	2.7	2.6		2.4		yes															
AML20	BM	0.32 (c.1	0.38 (c.8	0.47 (c.2	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt	46,XX	2.2	1.1	2.4	1.3		yes															
AML21	BM	wt	wt	0.47 (c.2	0.49 (IDH	wt	wt	0.44 (NR/	wt	wt	wt	0.03 (SF3	wt	wt	wt	wt	wt	wt	wt	wt	47,XX,+8[12]/45,XX,-7[4]/46,XX[1	3.0	0.7	3.3	3.1		yes															
AML22	PB	wt	wt	wt	wt	0.03 (c.3	wt	wt	wt	wt	wt	0.52 (SR	wt	wt	wt	wt	wt	wt	wt	wt	6,XY,t(11;22)(q23;q13)[10]/46,XY[12] KMT2A-EP300	0.8	0.8	1.3		yes	yes									0.34	0.06	1.12	1.03	2.94	2.58
AML23	BM	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK							autologous			0.87	0.38	1.13	1.12							
AML24	BM	ITD	0.43	0.36	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK							allogenic	0.90	0.68					1.445135							
AML25	BM	ITD	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK							autologous			0.59	0.27	1.07	0.94							
AML26	BM	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK							allogenic	1.43	1.31												
AML27	BM	0.26, 0.12	0.43	0.43	wt	wt	wt	0.05 (KR/	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	nd	1.8	2.9	1.5	1.0			allogenic	0.80	0.24					1.348404							
AML28	BM	ITD	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	+8							allogenic	1.11	1.03												
AML29	BM	0.44	wt	wt	wt	0.48	wt	wt	wt	0.48	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK	2.5	2.1	1.9	1.7			allogenic	1.11	0.41												
AML30	PB	0.47	0.48	0.48	0.43	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK							allogenic	0.88	0.35												
AML31	BM	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK							allogenic	1.20	0.21												
AML32	PB	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	46,XX,t(9;11)(p22;q23)	2.6		2.0												0.29	0.51	0.42	1.23	3.61	2.68
AML33	PB	ITD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	46,XY,t(7;11)(p15;p15)	3.4		3.6												0.77	0.29	0.90	1.06	1.34	1.70
AML34	BM	0.73	wt	wt	0.34	wt	wt	0.03	wt	wt	wt	wt	wt	wt	wt	nd	nd	nd	nd	nd	47,XY,t(3;5)(q23;q33),+8[10]	2.8		2.8												0.34	0.33	1.20	1.09	1.31	1.04
AML35	BM	wt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	46,XX,t(9;11)(p22;q23)[8]	3.1		3.5												0.05	0.24	0.25	1.89	1.34	1.01
AML36	PB	ITD	wt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6,XX,t(3;5)(q23;q33)[7]/46,XX[3]	3.7		2.2												0.29	0.24	1.17	1.34	1.12	1.31
AML37	BM	wt	wt	nd	nd	nd	nd	nd	nd	nd	nd	nd	wt	nd	nd	nd	nd	nd	nd	nd	46,XY,t(9;11)(p22;q23)	1.6		1.5												0.21	0.41	0.52	1.27	2.55	1.96
AML38	BM	wt	wt	nd	nd	nd	nd	nd	nd	nd	nd	nd	wt	nd	nd	nd	nd	nd	nd	nd	46,XX,t(9;11)(p22;q23)	2.1		1.6												0.24	0.63	0.51	1.10	1.04	1.10
AML39	PB	0.23	wt	0.45	wt	0.5	wt	0.06	wt	wt	wt	0.34	wt	wt	wt	wt	wt	wt	wt	wt	46,XX,t(10;17;15;21)(p11;q27?4;q15;q22)[4]/ 46,XX,t(1;12)(p13;p1															0.10	0.17	0.26		1.04	0.89
AML40	BM	ITD	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd															0.10	0.03		1.90	3.71	2.02
AML41	BM	wt	wt	wt	wt	wt	wt	0.48	wt	wt	wt	wt	wt	wt	wt	0.05	wt	wt	wt	wt	t(3,12)															0.77			0.92	0.98	1.17
AML42	BM	ITD	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	+8															0.31			0.67	0.92	0.59
AML43	PB	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	45,XY,-7,del(12)(p11p12)[7]/46,XY[3]															0.53	0.40	0.38	1.09	0.70	0.73
AML45	PB	wt	wt	wt	wt	wt	0.43	wt	wt	0.59	0.22	wt	wt	wt	wt	wt	wt	wt	wt	wt	47XY,+21	2.277	3.02	2.058	1.9																	
AML46	PB	wt	wt	wt	wt	wt	wt	wt	wt	0.44	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	6,XY,?inv(2)(p?21q?21),del(5)(p13p15),-7[10]	2.487		2.685	1.15																			
AML48	PB	ITD	wt	nd	nd	nd	nd	nd	nd	nd	pos	nd	nd	nd	nd	nd	nd	nd	nd	nd	complex															0.20	0.07	0.09	3.61	0.46	0.43
AML49	PB	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK															0.67	0.25		0.98	1.35	1.57
AML50	PB	0.26 (c.1	0.36 (c.8	0.49 (c.2	0.12 (IDH	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	46, XY													0.988189	0.01	0.03	0.63	1.87	1.45	1.25
AML51	PB	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd															0.08	0.21	0.72	0.90	0.87	0.73
AML52	BM	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK							autologous			1.01	0.61	0.76		0.961165						
AML53	BM	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	complex							autologous			0.79	0.67	1.47	1.59							
AML54	BM	0.13	0.4	0.5	0.4	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	NK							autologous			0.54	0.62	1.70	0.96	1.12037						
AML55	PB	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK							autologous			0.75	0.45	1.36								
AML56	PB	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK							autologous			0.71	0.27	1.10	1.44							
AML57	BM	wt	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	NK							autologous			0.94	0.86	1.03	1.28							
AML58	PB	ITD	cyt	nd	nd	nd	nd	nd	nd	nd	nd	nd	mut	nd	nd	nd	nd	nd	nd	nd	NK							autologous			0.66	0.55	1.29	1.16							
AML59	PB	wt		0.43	0.51	wt	0.46	wt	wt	0.06, 0.1	wt	wt	wt	0.31	wt	wt	wt	wt	wt	wt	NK	3.072		2.126	1.46																	
AML60	PB	wt		wt	wt	wt	wt	0.32(NR/	0.32 (c.13	0.43 (c.85	wt	wt	wt	0.48 (c.47	0.45 (BCC	0.34 (c.2	0.48 (c.13	9T>C)		NK	2.847	1.01	2.322	0.6																			
AML61	BM	wt		wt	0.48	wt	0.36	wt	wt	wt	wt	0.44, 0.0	wt	wt	wt	wt	wt	wt	wt	wt	NK	2.742	2.13	3.181	3.11																	
AML62	BM	wt		wt	wt	wt	0.47	wt	wt	wt	0.02	wt	0.55 (SR	0.34	wt	wt	wt	wt	wt	0.23	NK	3.052	2.4	3.753	2.62																	

Supplemental table 2. GSEA terms

Supplemental Table 2. GSEA terms					
follow link to MSigDB	**SIZE**	**ES**	**NES**	**NOM p-val**	**FDR q-val**
AML 2					
HSC SIGNATURE (EPPERT ET AL 2011)	86	-0.44	-2.50	0.00	0.00
LSC SIGNATURE (EPPERT 2011)	32	-0.29	-1.20	0.20	0.25
VALK_AML_CLUSTER_10	ns				
NPMCYT UP IN AML CD34 (WOOLTHUIS ET AL 2011)	94	-0.33	-1.93	0.00	0.00
MEISSNER_NPC_HCP_WITH_H3K4ME3_AND_H3K27ME3	101	-0.34	-1.92	0.00	0.07
GMP_DOWN (NOVERSHTERN ET AL 2011)	37	-0.32	-1.47	0.07	0.09
REACTOME_TRANSCRIPTIONAL_REGULATION_OF_PLURIP(	17	-0.64	-2.35	0.00	0.00
VALK_AML_CLUSTER_5	25	0.53	2.96	0.00	0.00
HALLMARK_INTERFERON_GAMMA_RESPONSE	ns				
GOCC_MHC_CLASS_I_PROTEIN_COMPLEX	5	0.54	1.46	0.04	0.36
BIOCARTA_MHC_PATHWAY	10	0.49	1.89	0.00	0.06
GOMF_MHC_PROTEIN_BINDING	19	0.30	1.60	0.08	0.27
AML 22					
HSC SIGNATURE (EPPERT ET AL 2011)	ns				
LSC SIGNATURE (EPPERT 2011)	ns				
VALK_AML_CLUSTER_10	25	-0.47	-2.14	0.00	0.01
NPMCYT UP IN AML CD34 (WOOLTHUIS ET AL 2011)	ns				
MEISSNER_NPC_HCP_WITH_H3K4ME3_AND_H3K27ME3	96	-0.33	-2.50	0.00	0.00
GMP_DOWN (NOVERSHTERN ET AL 2011)	ns				
REACTOME_TRANSCRIPTIONAL_REGULATION_OF_PLURIP(	17	0.64	1.59	0.01	0.11
VALK_AML_CLUSTER_5	29	0.56	1.62	0.02	0.10
HALLMARK_INTERFERON_GAMMA_RESPONSE	170	0.50	1.82	0.00	0.00
GOCC_MHC_CLASS_II_PROTEIN_COMPLEX	7	0.97	2.05	0.00	0.00
REACTOME_MHC_CLASS_II_ANTIGEN_PRESENTATION	98	0.46	1.62	0.00	0.10
BIOCARTA_MHC_PATHWAY	10	0.70	1.67	0.00	0.08
GOMF_MHC_PROTEIN_BINDING	17	0.71	1.94	0.00	0.01
AML 5					
HSC SIGNATURE (EPPERT ET AL 2011)	84	-0.39	-1.28	0.07	0.24
LSC SIGNATURE (EPPERT 2011)	31	-0.48	-1.44	0.01	0.07
VALK_AML_CLUSTER_10	27	-0.55	-1.72	0.00	0.05
NPMCYT UP IN AML CD34 (WOOLTHUIS ET AL 2011)	ns				
MEISSNER_NPC_HCP_WITH_H3K4ME3_AND_H3K27ME3	91	-0.48	-1.63	0.00	0.11
GMP_DOWN (NOVERSHTERN ET AL 2011)	37	-0.55	-1.70	0.00	0.00
REACTOME_TRANSCRIPTIONAL_REGULATION_OF_PLURIP(	18	-0.51	-1.45	0.02	0.29
VALK_AML_CLUSTER_5	ns				
HALLMARK_INTERFERON_GAMMA_RESPONSE	ns				
GOCC_MHC_CLASS_II_PROTEIN_COMPLEX	7	0.50	1.48	0.05	0.36
BIOCARTA_MHC_PATHWAY	10	0.51	1.66	0.00	0.14
GOMF_MHC_PROTEIN_BINDING	12	0.50	1.63	0.08	0.28

Supplemental Figure Legends

Supplemental figure 1. JNJ-75276617 impairs long-term proliferation of primary acute myeloid leukemia blasts under liquid culture conditions.

A. Schematic visualization of the JNJ-75276617 inhibitor drug screen in primary AML patient samples. B. Schematic overview showing gating strategy of the menin-KMT2A inhibitor drug screen. C. Dose-dependent effects on cell viability upon treatment of primary AML samples with JNJ-75276617 under liquid culture conditions. Statistical analysis by unpaired Student's t test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplemental figure 2. JNJ-75276617 impairs long-term proliferation of primary acute myeloid leukemia blasts under MS5 coculture conditions.

Dose-dependent effects on cell viability upon treatment of primary AML samples with JNJ-75276617 under MS5 co-culture conditions. Statistical analysis was performed by by an unpaired Student's t test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplemental figure 3. Effects of JNJ-75276617 on long-term proliferation in distinct genetic acute myeloid leukemia subgroups.

A. Boxplots showing AUC values of the proliferation of *NPM1c*, *DNMT3A*, *FLT3*, *RUNX1*, *IDH1*, *TET2* and *CEBPA* mutant vs wild type samples on LQ day 7 and 14 and MS5 day 7 and 14. B. Heatmap of AUC values at day 7 for liquid (LQ) culture conditions and MS5 coculture conditions in primary AML patient samples with various mutations. Heatmap showing expression of CD11b normalized to control after treatment with JNJ-75276617 for LQ and MS5 culturing conditions. C. Paired analysis

of AUC values calculated for the effect of JNJ-75276617 inhibitor treatment on liquid (LQ) and MS5 co-cultured primary AML patient cells on day 7 and 14 (n=14, biological replicates day 7 and n=15, biological replicates day 14). D. Correlation plot comparing AUC values from MS5 day 7 and LQ day 7 (n=14). Statistical analysis by unpaired Student's t test or Simple Linear Regression. * P < 0.05, ** P < 0.01, *** P < 0.001.

Supplemental figure 4. JNJ-75276617 drives differentiation of primary acute myeloid leukemia blasts.

A. Lineplots showing the MFI of CD11b normalized to the DMSO control for LQ day 7 (n=12). Black curves represent samples in which JNJ-75276617 also blocked proliferation, red curves represent samples in which proliferation was less effected by JNJ-75276617 treatment. B. Comparison between AUC and CD11b expression (normalized to DMSO control) identifying 4 groups: proliferation, differentiation and proliferation, differentiation only, and weak responders. Red dots represent samples in which proliferation was less effected by menin-KMT2A inhibition. C. Barplots depicting the MFI of CD11b (normalized to DMSO control) of CB KMT2A-AF9 on LQ day 7. D. Cytospins of DMSO and 3.0 µM JNJ-75276617 inhibitor treated CB KMT2A-AF9 cells at LQ day 7. E. Boxplots showing AUC values of the normalized MFI of CD11b of *NPM1c*, *DNMT3A*, *FLT3*, *RUNX1*, *TET2, CEBPA* and KMT2A-rearr. mutant vs wild type on LQ day 7 and 14 and MS5 day 7 and 14. NA = not assessed due to little number of samples in testing conditions. Statistical analysis by unpaired Student's t test. * P < 0.05, ** P < 0.01, ** P < 0.001.

Supplemental figure 5. Quantitative proteome analyses links menin-KMT2A inhibitor sensitivity to more committed L-GMP-type acute myeloid leukemias in *NPM1*^{wt} only

A. Volcano plot of the pearson correlation coefficient and log10 p-values of proteome dataset versus AUC values of LQ day 7. B. Dotplot of gene set enrichment analysis (GSEA) signatures enriched in sensitive and less sensitive primary AML samples at LQ day 7. C. Highlighted gene set enrichment signatures from panel B. D. Volcano plot of the pearson correlation coefficient and log10 p-values of proteome dataset versus AUC values of MS5 day 7. E. Highlighted gene set enrichment signatures from panel B. F. Protein expression of HOXA10 and MEIS1 in *NPM1*^{wt} and *NPM1c* AML samples. Red dots and annotated samples are *NPM1*^{wt} AMLs sensitive to menin-KMT2A inhibition.

Supplemental figure 6. Quantitative proteome analyses links menin-KMT2A inhibitor sensitivity to more committed L-GMP-type acute myeloid leukemias in *NPM1*^{wt} only

A. Comparison between AUC and HOXA10, MEIS1, or IGF2BP2 protein expression in primary AML samples cultured for 7 days under liquid conditions. Red dots *NPM1c*, black dots *NPM1*^{wt}. Annotated samples are *NPM1*^{wt} AMLs sensitive to menin-KMT2A inhibition. B. Comparison between AUC and HOXA10, MEIS1, or IGF2BP2 protein expression in primary AML samples cultured for 7 days under liquid conditions. Red dots annotate *NPM1c*, black dots *NPM1*^{wt}. C. Dotplots showing GSEA signatures enriched in sensitive and less sensitive *NPM1*^{wt} only AMLs. D. Comparison between AUC values in GMP-like, Mixed and HSC-like groups of LQ day 7. E. Dotplot showing GSEA signatures enriched in NPM1c/DNMT3Amut AML samples from the TCGA

dataset. F. Dotplot showing GSEA signatures enriched in NPM1c/DNMT3Amut AML samples from the proteome dataset. Statistical analysis by unpaired Student's t test. * P < 0.05, ** P < 0.01, ** P < 0.001.

Supplemental figure 7. HOXA9 expression upon menin-KMT2A inhibition

A. Barplots showing relative mRNA expression of *MEIS1, IGF2BP2 and HOXA9* in four-day JNJ-75276617 inhibitor-treated primary AML patient samples normalized to DMSO of AML36. Heatmap showing effect on proliferation (AUC) and induction of CD11b expression (normalized to DMSO control). B. Barplots showing relative mRNA expression of *MEIS1* and *IGF2BP2* in four-day JNJ-75276617 inhibitor-treated OCI-AML3 and MOLM13 cells normalized to DMSO. C. Barplots depicting absolute cell counts in OCI-AML3 and MOLM13 cells with EV-GFP or MEIS1-GFP treated with DMSO or 0.3 µM JNJ-75276617 inhibitor (representative of n=3). D. Relative mRNA expression of *IGF2BP2* in four-day JNJ-75276617 inhibitor-treated OCI AML3 cells with EV-GFP or IGF2BP2-GFP. E. Fold change DAPIneg/GFP+ counts of OCI-AML3 cells with EV-GFP or IGF2BP2-GFP treated with DMSO, 0.03, 0.3 or 3.0 µM JNJ-75276617 inhibitor for seven days. F. Relative mRNA expression of *MEIS1* and *BMI1* in four-day JNJ-75276617 inhibitor-treated OCI AML3 cells with EV-GFP or IGF2BP2-GFP. Error bars represent mean ± standard error of the mean (SEM). Statistical analysis by unpaired Student's t test or Simple Linear Regression. * P < 0.05, ** P < 0.01, *** P < 0.001.

Supplemental figure 8. GO analyses of loci with altered H3K4me3 marks.

A. GSEA signatures of a ranked fold change list of H3K4me3 expression changes after menin-KMT2A inhibition of AML2, AML22 and AML5. Processes associated with H3K4me3 downregulation are shown. B, GSEA as in A, but now processes associated

with H3K4me3 upregulation are shown. C. Dotplots showing gene ontology (GO) terms of genes with a fold change (FC)<-0.585 in H3K4me3 marks after treatment. D. As in C, but now dotplots for GO terms for genes with a FC>0.585 in H3K4me3 marks are shown.

Supplemental figure 9. GO analyses of loci with altered H3K4me3 and H3K27ac marks.

A. GSEA signatures of a ranked fold change list of H3K27ac expression changes after menin-KMT2A inhibition of AML2, AML22 and AML5. Processes associated with H3K27ac upregulation are shown. B. Dotplot of gene ontology (GO) terms of genes that have upregulated H3K4me3 expression in OCI-AML3 cells after JNJ-75276617 inhibitor treatment. FC > 0.585.

Supplemental figure 10. Menin-KMT2A inhibition drives HLA expression in a MEIS1-independent but CIITA-dependent manner in the case of MHC-II.

A. Relative mRNA expression of *HLA-A, HLA-DR and CIITA* in four-day JNJ-75276617 inhibitor-treated primary AML patient samples normalized to DMSO of AML36 or AML37 (*HLA-A*). Heatmap showing effect on proliferation (AUC) and induction of CD11b expression (normalized to DMSO control). B. Relative mRNA expression of *HLA-A, HLA-DR and CIITA* in four-day JNJ-75276617 inhibitor-treated primary AML patient samples normalized to DMSO of AML36 or AML37 (*HLA-A*). Samples not ranked based on AUC value. C. Correlation plots comparing fold changes of *HLA-DR* and *CIITA* mRNA expression after menin-KMT2A inhibition. D. Correlation plots comparing fold changes of *HLA-DR* and *MEIS1* mRNA expression after menin-KMT2A inhibition. E. Barplot showing MFI of HLA-DR expression in MV4-11 cells with EV-GFP

or MEIS1-GFP treated for four days with DMSO or JNJ-75276617 inhibitor (n=3). Statistical analysis by unpaired Student's t test. * P < 0.05, ** P < 0.01, *** P < 0.001.

Supplemental figure 11. Menin inhibition enhances T cell cytotoxicity

A. Barplots showing relative viable cell counts from the experiment shown in Figure 7C, determined by Annexin-V and Zombie NIR™, of 8 primary AML samples grown in the absence (0:1) or in the presence (5:1) of allogeneic T cells, whereby cell were pre-treated with DMSO or 0.3 µM JNJ-75276617. B. Primary AML samples were treated for four days with JNJ-75276617 or DMSO controls after which allogeneic T cells were added for an additional 3 days. Viable T cell counts are shown. C. $CD4^+$ percentage in viable T cells of primary AML sample cultures with T cells (enriched). D. Relative CD69 MFI expression in viable T cells from primary AML sample cultures without T cells (depleted) or with T cells (enriched). Statistical analysis by unpaired Student's t test. * P < 0.05, ** P < 0.01, ** P < 0.001.

Supplemental Methods

Primary samples

AML blasts from peripheral blood or bone marrow from untreated patients were studied after informed consent and the protocol was approved by the Medical Ethical Committee of the University Medical Center Groningen, The Netherlands, in accordance with the Declaration of Helsinki. Mononuclear cells (MNCs) were isolated via Lymphoprep™ separation and cryopreserved. Next Generation Sequencing was performed to obtain mutation status of primary AML cells using the TruSight Myeloid Sequencing Panel (Illumina) or exome sequencing. Neonatal cord blood (CB) samples

were obtained from healthy full-term pregnancies at the obstetrics departments at the Martini Hospital and University Medical Center Groningen.

Cell culture

The AML cell lines OCI-AML2 (DSMZ: ACC-99) and OCI-AML3 (DSMZ: ACC-582) were cultured in alpha MEM (Lonza). Cell line KG1A (DSZM: ACC-421) was cultured in IMDM (Lonza) and IMS-M2 cells were cultured in RPMI 1640 (Lonza) all with 20% fetal bovine serum (FCS, Sigma-Aldrich) and 1% penicillin/streptomycin (P/S, Gibco™). MV411 (DSMZ: ACC-102), MOLM13 (DSMZ: ACC-554), K562 (DSMZ: ACC-10), THP1 (DSMZ: ACC-16) and HL60 (DSMZ: ACC-3) cells were cultured in RPMI 1640 with 10% FCS and 1% P/S. CB MLL-AF9 cells[1] were cultured in Gartner's medium supplemented with IL-3 (Sandoz), SCF (Novus Biologicals) and FLT-3L (Amgen) (10 ng/ml each). MS5 murine stromal cells (DSMZ: ACC-441) were cultured in alpha-MEM (Lonza) with 10% FCS and 1% P/S. All cell cultures were kept at 37°C and 5% CO_2. For all menin-KMT2A interaction inhibition experiments inhibitor JNJ-75276617 was provided by Janssen Biologics BV.

Menin inhibitor screen in primary AML samples

Cryopreserved MNCs of AML patients were either cultured in liquid culture supplemented with G-CSF (Amgen), N-Plate (TPO)(Amgen) and IL-3 (Sandoz) (all 20 ng/mL) or co-cultured in Gartner's medium supplemented with cytokines on MS5, which were confluent plated on 0.1% gelatin-coated 48 wells plates and pre-treated with Mitomycin C. 150.000 MNCs per well were plated per well, and after two days recovery cells were treated with DMSO or 0.03, 0.30 and 3.00 µM JNJ-75276617 inhibitor for 14 days. Fresh medium and inhibitor were added at day 7 after demi-

population of the cells. On day 7 and 14 cells were stained with CD45-PECy7 (BioLegend; 304016), CD117-PE (ImmunoTools; 21271174X2), CD11b-FITC (ImmunoTools; 21279113X2), and DAPI (ThermoScientific) in a 96 wells plate, and were incubated for 30 min at 4°C. Fluorescence measurements were taken using a MACSQuant® X Flow Cytometer (Miltenyi Biotec)

Menin inhibitor screen in cell lines

AML cell lines were treated with DMSO or 0.03, 0.30 and 3.00 µM JNJ-75276617 inhibitor for 14 days. Every 2 days fresh medium and menin inhibitor was added after demi-population of the cells.. On day 7 and 14 cells were stained with DAPI in a 96 wells plate and analysed using a MACSQuant® X Flow Cytometer.

Menin inhibitor screen in healthy samples

Healthy MNCs were isolated from CB by a density gradient using Lymphoprep™ (STEMCELL™ Technologies). Stem cells were isolated using the CD34 Microbead Kit (Miltenyi). After 24h recovery in Stemline® II hematopoietic medium (Merck; #S0192) supplemented with 1% P/S, SCF, FLT3-L and N-plate (TPO)(Amgen)(all 100 ng/mL), $CD34^+$ CB cells were treated with DMSO or 0.03, 0.30 and 3.00 µM JNJ-75276617 inhibitor for 14 days in liquid culture with G-CSF (Amgen), N-Plate and IL-3 (all 20 ng/mL) or co-cultured on MS5 in Gartner's medium without supplementation of cytokines.

Flow cytometry data analysis inhibitor screen

All flow data was analysed using Flow Jo™ (BD BioSciences). Counts, percentages and Median Fluorescent Intensities were exported for further analysis. To calculate

area under the curve (AUC) values $DAPI^-/CD45^{dim}$ (primary samples) or $DAPI^-$ (cell lines) counts were normalized to the DMSO control. Next, AUC was calculated using trapezoid rule integration computed by the trapz() function in the R package caTools. CD11b MFI values were normalized to DMSO control.

Sample preparation and mass spectrometry analysis

Cryopreserved primary AML patient samples were thawed as described in the 'Menin inhibitor screen in primary AML samples' section. After thawing MNCs were isolated for $CD34^+$ (*NPM1*^{wt}), $CD117^+$ (*NPM1*^{cyt}) or depleted for CD3 (samples with low CD34/CD117%; CD3 MicroBeads, human; Mitenyi Biotec) by autoMACS (Miltenyi Biotec). 1.5E6 cells were snap frozen in liquid nitrogen. In addition, three peripheral blood $CD34^+$ healthy control samples were taken along as well.

Mass spectrometry analysis

Cell lysis and MS sample preparation for DIA SingleShot analysis

Cell lysis was performed in PreOmics LYSE buffer and samples were incubated at 95°C for 10 minutes.[2] Cell extracts were sonicated and protein concentrations were determined using a BCA protein assay. Complete cell extracts were digested employing 50 µl of PreOmics DIGEST reagent at 37°C overnight. Tryptic peptides were desalted by solid phase extraction using PreOmics CARTRIDGE according to the manufacturer's instructions. After lyophilization and resuspension in 0.1% formic acid, 1.7 µg of peptide per samples were applied to mass spectrometry analysis.

MS sample preparation for DDA Peptide Library generation

10 µg peptide of each sample (except of samples H1603 and H1604) were pooled and lyophilized. The pooled peptide sample was then subjected to pre-fractionation by high pH reversed phase chromatography[2] for deep proteome profiling. Briefly, peptides were reconstituted in 10 mM ammonium formate (pH 10, buffer A) and loaded onto an XBridge C18, 200 × 4.6 mm analytical column (Waters) operated with an UltiMate 3000 HPLC and UHPLC Systems (Thermo Fisher). Peptides were separated by applying a segmented gradient with increasing acetonitrile concentration from 7% to 30% buffer B (buffer A supplemented with 80% acetonitrile) over 15 min followed by a 5 min gradient to 55% buffer B. The collected fractions were combined in a concatenated way to generate a total of 10 fractions. Pooled fractions were then desalted via 100 mg SepPack C18 columns (Waters), lyophilized and reconstituted in 0.1% formic acid. For subsequent MS analysis, 1.7 µg of peptide per fractionated samples were applied in triplicates.

Mass spectrometric analysis

LC-MS/MS experiments were performed on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific) equipped with an Easy nLC-1200 UPLC system (Thermo Fischer Scientific). Samples were loaded with an auto sampler onto a 40 cm fused silica emitter (New Objective) packed in-house with reversed phase material (Reprusil-Pur C18-AQ, 1.9 µm, Dr. Maisch GmbH) at a maximum pressure of 1150 bar. The bound peptides were eluted over 125 min run time and sprayed directly into the mass spectrometer using a nano electrospray ion source (ProxeonBiosystems). For the analysis of single shot samples, the mass spectrometer was operated in the data independent mode (DIA). The DIA-MS method consisted of a survey scan at 120,000

resolution from 350 to 1650 m/z. DIA windows (ms/ms scans) were acquired at 30,000 resolution and the number of windows was set to achieve 4 data points per peak.
For the analysis of peptide library samples, the mass spectrometer mass spectrometer was operated in a data-dependent acquisition mode to automatically switch between full scans (resolution R=60.000) and the acquisition of HCD fragmentation spectra (MS/MS mode) of the ten most abundant peptide ions in the Orbitrap mass analyzer (resolution R=15.000).

Mass spectrometry data processing

All raw files acquired in this study were collectively processed with the Dia-NN software suite (version 1.7.10) for peptide/protein identification and quantification using a human Uniprot database (downloaded February 2020).[3] Default parameters were used. Furthermore, the precursors identified at a Q-value below 1% were used to infer protein groups with an adapted version of the ID Picker algorithm.[4] The corresponding protein group intensities were computed with an adapted version of the maxLFQ algorithm[5] taking all assigned precursors into account. In addition to the LFQ intensities, iBAQ values were computed using the summed mean raw precursor intensities per peptide and the number of all theoretical unique tryptic peptides per protein group.[6] Copy number estimates and protein concentrations were calculated with Perseus (v 1.6.0.2)[7] applying the proteomic ruler[8] approach on the iBAQ values. Over 15.000 proteins could be quantified (PXD030487).

LFQ proteome data analysis

Pearson correlations were performed using the calculated AUC and the LFQ proteome dataset. 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.

Heatmap and one minus pearson correlation clustering of *NPM1c* differentially expressed proteins were generated using the Morpheus tool from clue.io.

ChIP-seq with spike-in and data analysis

ChIP experiment

Chromatin immunoprecipitation was performed as described previously.[9] OCI AML3 cells were treated with 0.3 µM menin-KMT2A interaction inhibitors for 7 days and after cross linking spiked with drosophila chromatin. Primary AML samples were treated with 0.3 µM or 0.03 µM JNJ-75276617 inhibitor for 5 days and after cross linking spiked with mouse chromatin. The following antibodies were used: anti-H3K4me3 (C15200152; Diagenode), anti-H3K27ac (C15410196; Diagenode), anti-H3K27me3 (C15410195; Diagenode) and IgG (i8141, Sigma). Sequencing libraries were generated using the KAPA Hyper Prep Kit (Roche Sequencing and Life Sciences) according to manufacturer's protocol and sequenced on an Illumina NextSeq500 using default parameters.

Alignment

ChIP-rx data analysis was done as described in Orlando *et al.*, 2014.[10] In short, combined reference genomes were generated for human (hg38) and drosophila (dm6) or human (hg38) and mouse (mm10) with 'dm6_' or 'mm10_' suffix to avoid chromosome name duplications. Obtained paired-end reads were aligned to the metagenomes using Burrows-Wheeler Aligner (BWA)[11] with default settings. Generated SAM files were split in two resulting in a file containing reads that aligned to human chromosomes and a file containing reads that aligned either to drosophila

chromosomes or mouse chromosomes. Aligned reads were further processed using SAMtools.[12] Normalization factors were calculated per file as described in Orlando *et al.*, 2014.[10]

Visualization of tracks

To visualize the tracks bigwig files were generated by determining the total number of overlapping fragments at each position in the genome using BEDtools genomecov. The coverage was scaled using the calculated normalization factors. Subsequently, BedGraph files were converted to BigWig files using UCSC bedGraphToBigWig. Tracks were visualized using the Integrative Genomics Viewer.[13] Data is deposited at GSE237834.

Peak calling and further processing

Peaks were called using MACS2[14] with estimated fragment size and broad settings. To be able to compare coverage from different samples peaks were concatenated and merged per histone mark. For every track read counts were generated and the coverage was normalized using the normalization factor calculated before.

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

Table 2. Primers used for ChIP-qPCR		
Target	**Forward**	**Reverse**
CIITA -0.5	GAAAATGACAGGTGGGCCACTTATGATCTC	TCCCACACCAAATTGCCCTGAATTTCTC
CIITA -0.2	AACAGACTTTCTGTGCAACTTTCTGTCTTC	TGAACACCCTCTAATTTTACCACACTCCC
CIITA +1.7	TGCTTGGTTGCTCCACAGCCTG	CCCGCAGTTCTTTTTCCCTTTCACTTTC
HLA-DR -0.6	AAGCTCTTGGCCTGAGTTGA	CAGGCCATGGAGATTGTCTGA
HLA-DR -0.2	GTCTGTTCTGCCTCACTCCC	ATCCTAGCACAGGGACTCCA
HLA-DR +0.2	GGTGGAGTTCTTCCCTCACC	TGGCTTGTAGCAGGACCTTG

Supermix (Bio-Rad) on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad). Primer sequences are listed in table 1 and 2.

Generation of MEIS1-EGFP, IGF2BP2-IRES-EGFP and EGFP-MEN1 lentivectors

Generation of lentiviral pRRL SFFV MEIS1-EGFP was done by Genscript. The EGFP-MEN1 expression vector was constructed by amplifying the MEN1 cDNA sequence and EGFP sequence from K562 derived cDNA and pEGFP-C1 (Takara Bio Europe SAS), respectively. The IGF2BP2-IRES-EGFP expression vector was constructed by amplifying IGF2BP2 cDNA sequence and EGFP sequence from THP1-derived cDNA and pEGFP-C1, respectively. For the EGFP-MENIN1 vector, primers were designed to have 5' overhangs complementary to the fusion partners and the amplified products was ligated using Gibson assembly in EcoRI/BsrGI-cut pRRL-SFFV-IRES-EGFP. For the IGF2BP2-IRES-EGFP vector, the same strategy was followed and the amplified product was ligated in XhoI-cut pRRL-SFFV-IRES-EGFP. The construct sequence was verified by Sanger sequencing.

Table 1. Primers used for mRNA expression		
Target	**Forward**	**Reverse**
MEIS1	TCTGCCACCGGTATATTAGC	GAACGAGTAGATGCCGTGTC
IGF2BP2	TTCGAAACATCCCTC	CTGTGTCTGTGTTGACTTGTT
HLA-A2	TCCTGCTACTCTCGGGGGCT	CTCCCACTTGTGCTTGGTGG
HLA-DRA	CTGAGGACGTTTACGACTG	CACACCACGTTCTCTGTAG
CIITA	GGCTGGGATTCCTACACA	ACACTGTGAGCTGCCTTG
BMI1	GTTCCCTCCACCTCTTCTTG	GGCTCTTGCTGGTTCCATTC

Generation of CRISPR/Cas9 knockout lines

3xNLS-SpCas9 expression and purification

Gene editing was essentially performed as we described previously.[15] 3xNLS-SpCas9 was purified essentially according to the method described in Wu et al, 2019[16] with some modifications. In short: the vector pET-21a_3xNLS-SpCas9 was obtained from Addgene (Addgene, #114365) and transformed to Rosetta 2(DE3)pLysS competent cells (Novagen, Merck, Amsterdam, the Netherlands). One single colony was precultured overnight at 37 °C in 10 ml of LB medium containing Ampicillin (100 µg/ml) and Chloramphenicol (34 µg/ml) and subsequently diluted in 1 liter of TB medium containing Ampicillin, Chloramphenicol and 0,5 mM IPTG and grown overnight (16 hours) at room temperature with vigorously shaking. The next day, the culture was harvested, centrifuged for 20 minutes at 3000 g and the pellet was resuspended in 20 ml of Ni-NTA binding buffer (20 mM TRIS, 500 mM NaCl, 20 mM imidazole, 1 mM TCEP, pH 8.0), flash frozen in liquid nitrogen and stored at -20 °C.

For purification, the bacterial suspension was thawed and PMSF (1 mM final concentration) and lysozyme (final concentration of 0,5 mg/ml) was added and incubated at room temperature for 10 minutes with rotation. DNAse (Roche, Merck, Amsterdam, the Netherlands) was added (0,01 mg/ml final concentration) and the solution was incubated with rotation for another 30 minutes at room temperature. The lysate was cleared by centrifugation for 30 minutes at 20.000 g, 3 ml Ni-NTA agarose slurry (Qiagen, Venlo, the Netherlands) was added to the supernatant and rotated for 1 hour at 4 °C. The Ni-NTA bead containing lysate was divided over 3 Polyprep columns (Bio-Rad, Veenendaal, the Netherlands) and after settling the column, the beads were washed 2 times with 5 ml of Ni-NTA binding buffer. The protein was eluted

with 1,3 ml elution buffer (20 mM TRIS, 250 mM NaCl, 250 mM Imidazole, pH 8.0) per column and pooled. The eluate was diluted 10 times with SCX wash buffer (50 mM HEPES, 400 mM NaCl2, pH 7,4) to decrease the imidazole concentration and loaded onto a 5 ml HiTrap SP FF strong cation exchange column (Cytivia, Merck, Amsterdam, the Netherlands) with a flow rate of 5 ml/min. The column was washed with 50 ml SCX wash buffer and the protein was eluted with SCX elution buffer (50 mM HEPES, 700 mM NaCl2, pH 7,4) while collecting fractions of 0,5 ml. The A280 of the fractions was measured and the fractions containing the purified protein were pooled (4,5 ml total) and desalted in 3 portions of 1,5 ml over a 5 ml HiTrap desalting column (Cytivia, Merck, Amsterdam, the Netherlands) equilibrated with storage buffer (20 mM HEPES, 150 mM NaCL2, 10% glycerol, pH 7,4). The resulting purified and desalted protein was concentrated to 1 ml using an Amicon Ultra 15, 100.000 MWCO spin filter (Millipore, Merck, Amsterdam, the Netherlands), the concentration was measured and adjusted to 5 mg/ml. 20 µl portions were flash frozen in liquid nitrogen and stored at -80 °C for further use.

Guide RNA selection

The online platform Benchling (www.benchling.com) was used to design guide RNA sequences for CIITA. Two different gRNAs were selected based on high on-target and off-target scores. gRNA sequences are listed in table 3.

sgRNA preparation

sgRNA was made by *in vitro* transcription of a dsDNA PCR product. In short: a DNA template was made by oligo assembly using a set of three generic oligos (Sp6-forward, scaffold oligo and Sp6-reverse) and one guide specific oligo. PhusionII HF polymerase

(Thermo Scientific, Bleiswijk, the Netherlands) was used to amplify the DNA template. Final PCR products have the following sequence: 5'-GATCATTTAGGTGACACTATA(G)NNNNNNNNNNNNNNNNNNNGTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC-3', in which N stands for the specific guide sequence and (G) stands for the addition of an extra G when the guide sequence does not have a 5' G. The Sp6 priming sequence is underlined. The sequences of the oligos are listed in table 4. The template contains an Sp6 priming site to facilitate in vitro transcription using a HiScribe SP6 RNA synthesis kit (New England Biolabs, Ipswich, MA, USA). 300 ng template was used in an overnight reaction, which typically yielded 100-150 µg sgRNA. sgRNA was purified by a Monarch RNA cleanup kit (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions and stored at -80°C until use.

Procedure

RNP complexes were formed *in vitro* by adding 12 µg sgRNA to 20 µg spCas9 and incubation for 15 minutes at room temperature. MV4-11 cells (1 x106) were washed once with PBS and resuspended in 100 µl K562 electroporation buffer (88 mM KH2PO4, 14 mM NaHCO3, 12 mM MgCl2, 2 mM glucose and 6 mM ATP, pH 7,4), the RNP complex was added and transferred to a 2 mm gap width electroporation cuvette. The mixture was electroporated with an Amaxa I electroporation device (Lonza, Geleen, the Netherlands) using program D-23, immediately transferred to 4 ml fresh medium (RPMI1640 + 10 % FCS) and cultured for 3 days. Single cells were sorted in 96 well plates using a MoFlo XDP cell sorter (Beckman Coulter, woerden, the Netherlands) and incubated for 12 days whereafter plates were scored for the

presence of growing colonies. Approximately 12 clones per sgRNA were expanded in 24 well plates and gDNA was extracted from a part of the clone. gDNA spanning the expected cut site was PCR amplified using primers listed in table 5 and Sanger sequenced. Sequencing tracks were analyzed using the online available TIDE analysis tool (www.tide.nki.nl). Clones with lesions resulting in premature stop codons on both alleles were expanded and frozen. For experiments, clones were pooled again to avoid possible clonal differences unrelated to the knockouts.

Table 3: guide RNA targets			
Gene	**Guide**	**Exon**	**Target sequence**
CIITA	1	8	GAGATTCAGGCAGCTCAACG
	2	8	GAGCTGCCTGAATCTCCCTG

Table 4: sequences of the DNA template oligos for sgRNA production	
Oligo	Sequence
Sp6-forward	GATCATTTAGGTGACACTATAG
Scaffold oligo	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTAAACTTGCTATGCTGTTTCCAGCATAGCTCTTAAAC
Sp6-reverse	GCACCGACTCGGTGCCAC
CIITA (1)	GATCATTTAGGTGACACTATAGAGATTCAGGCAGCTCAACGGTTTAAGAGCTATGCTGGAAAC
CIITA (2)	GATCATTTAGGTGACACTATAGAGCTGCCTGAATCTCCCTGGTTTAAGAGCTATGCTGGAAAC

Table 5: primers used for sequencing of CRISPR/Cas9 targets		
Target	Forward	Reverse
CIITA exon 8	TAAGGCAGGGACTGTCAGGAAC	TGCAAGGATGCACACCAAAC

Flow cytometry analysis

Hetero- and homozygous *CIITA* knockout MV4-11 cells were treated with 0.03, 0.30 or 3.00 µM JNJ-75276617 inhibitor for four days after which 100.000 cells were stained for HLA-DR-PE (Biolegend; 307606) and incubated for 30 min at 4°C. Fluorescence measurements were taken using a MACSQuant® X Flow Cytometer (Miltenyi Biotec).

Allogeneic and autologous killing assay

T-cell isolation and activation

Allogeneic T cells were isolated from cord blood or peripheral blood samples from healthy or allogeneic donors. MNCs were isolated by a density gradient using Lymphoprep™ (STEMCELL™ Technologies) and $5x10^6$ MNCs were plated into 6 well plates. Next, the non-adherent cells were collected and T-cells were isolated with the Pan T Cell Isolation Kit (Miltenyi Biotec) using the autoMACS (Miltenyi Biotec). To activate the isolated T-cells the cells were cultured in RPMI medium supplemented with 10% FCS, 1% P/S, IL-2 (6000 IU/ml) and Gibco Dynabeads Human T-Activator CD3/CD28 beads for 9 days. Autologous T cells were isolated from MNCs of primary AML patient samples using the Pan T Cell Isolation Kit as described above. After a 24h recovery these T-cells were activated for 3 days using IL-2 (6000 IU/ml) and Gibco Dynabeads Human T-Activator CD3/CD28 beads.

Preparation AML cells

MV411 and MOLM13 cells were cultured as described above and treated with 0.1 µM or 0.3 µM menin inhibitor for 4 days. Primary AML samples were thawed as described in section 'Menin inhibitor screen in primary AML samples' and cultured in α-MEM with 20% FCS and 1% P/S supplemented with G-CSF (Amgen), N-Plate (TPO)(Amgen) and IL-3 (Sandoz) (all 20 ng/mL). After one recovery day the cells were treated with 0.3 µM menin inhibitor for 4 days. After 4 days the cells were collected for T-cell killing and stained for HLA-DR-PE (Biolegend; 307606) after which they were incubated for 30 min at 4°C. Fluorescence measurements were taken using a MACSQuant® X Flow Cytometer (Miltenyi Biotec).

Allogeneic killing assay

To start the allogeneic killing assay, PBMCs and activated T-cells were washed with serum free medium and stained with CellTrace™ Violet Cell Proliferation dye (ThermoFisher) for 20 min at 37ºC. Next, the cells were washed twice with serum containing medium and effector to target cell ratios were determined. The immune cells were loaded on top of the AML cells and after 3 days cells were stained with a staining mix containing Ca2+ buffer (BD Biosciences), AnnexinV-APC (Biolegend), and Zombie NIR (Biolegend). All flow cytometry was done using the NovoCyte Quanteon Flow Cytometer Systems 4 Lasers (Agilent).

Autologous killing assay

60.000 $CD3^+$-depleted primary AML cells were seeded with or without 10.000 autologous T cells (T-enriched or T-depleted, respectively) in RPMI with 10% FCS and 1% P/S. Cells were then treated with DMSO (WAK-Chemie Medical GmbH) or JNJ-75276617 0.3 µM for 4 days. Afterwards, the cells were stained with a staining mix containing Ca2+ buffer (BD Biosciences), AnnexinV-APC (Biolegend; 640941), Zombie NIR (Biolegend; 423106), CD3-BV785 (Biolegend; 317330) and CD45-PE (Biolegend; 304008). Simultaneously, 100.000 cells were stained for HLA-A,B,C-APC (Biolegend; 311410) and HLA-DR,DP,DQ-FITC (Biolegend; 361706). All flow cytometry measurements were done using the NovoCyte Quanteon Flow Cytometer Systems 4 Lasers (Agilent).

Data analysis allogeneic and autologous killing

All flow data were analyzed with NovoExpress Software (Agilent). After excluding immune cells based on cell trace violet, viable AML cells were gated out by AnnexinV-

APC and Zombie NIR. Counts were exported for further analysis. Fold change was calculated by normalizing all other treatments to their own controls, namely 0:1 E:T ratio, DMSO-treated cells and DMSO-treated T-depleted conditions in cell line killing, primary AML allogeneic killing, and primary AML autologous killing respectively.

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 $p \leq 0.05$.

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