SUPPLEMENTARY APPENDIX

Targeting BRD4 in acute myeloid leukemia with partial tandem duplication of the MLL gene

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Targeting BRD4 in acute myeloid leukemia with partial tandem duplication of the *MLL* gene

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Statistical analysis

Student's t-test, IC_{50} evaluation, the overall survival analysis (log-rank-test), and one-way ANOVA analysis were performed using GraphPad Prism as previously described (Bill M, et al. *Clin Cancer Res.* 2020;26(3):669-678).

Supplementary Tables

Supplementary Table 1. List of genes that are upregulated/downregulated by an aberrant BRD4 binding in *MLL*-PTD AML cells.

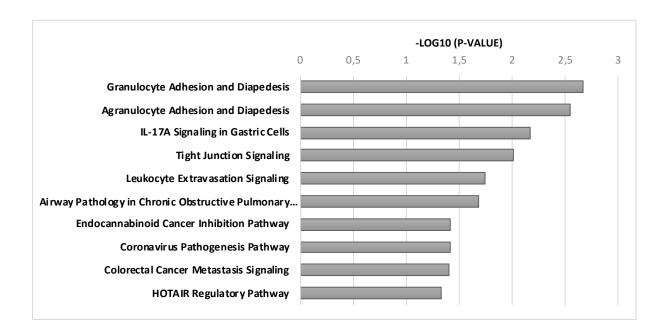
Upregulated genes				
Gene	Name	Type of gene		
ACTR3C	actin related protein 3C	Protein coding		
ADAMDEC1	ADAM like decysin 1	Protein coding		
ANG	angiogenin	Protein coding		
ASGR2	asialoglycoprotein receptor 2	Protein coding		
AVPI1	arginine vasopressin induced 1	Protein coding		
B3GNT8	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8	Protein coding		
C10orf91	chromosome 10 open reading frame 91	ncRNA		
C10orf95	chromosome 10 open reading frame 95	ncRNA		
C17orf59	BLOC-1 related complex subunit 6	Protein coding		
CCDC122	Coiled-coil domain-containing protein 122	Protein coding		
CCDC78	coiled-coil domain containing 78	Protein coding		
CD19	CD19 molecule	Protein coding		
CDK9	cyclin dependent kinase 9	Protein coding		
CLDN7	claudin 7	Protein coding		
CLDN9	Claudin 9	Protein coding		
CTXN1	cortexin 1	Protein coding		
CXCL1	C-X-C motif chemokine ligand 1	Protein coding		
DIRAS1	DIRAS family GTPase 1	Protein coding		
DUSP28	dual specificity phosphatase 28	Protein coding		
EFHB	EF-hand domain family member B	Protein coding		
EPS8L2	EPS8 like 2	Protein coding		
ESPNL	espin like	Protein coding		
ETV2	ETS variant 2	Protein coding		
FAM109B	Sesquipedalian-2	Protein coding		
FCN1	ficolin 1	Protein coding		
FDXR	ferredoxin reductase	Protein coding		

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	TPSAB1	tryptase alpha/beta 1	Protein coding

TRIOBP	TRIO and F-actin binding protein	Protein coding
TYROBP	TYRO protein tyrosine kinase binding protein	Protein coding
UNC5B	unc-5 netrin receptor B	Protein coding
USHBP1	USH1 protein network component harmonin binding protein 1	Protein coding
USP44	ubiquitin specific peptidase 44	Protein coding
WDR25	WD repeat domain 25	Protein coding
ZC3H3	zinc finger CCCH-type containing 3	Protein coding
ZFP36	ZFP36 ring finger protein	Protein coding
ZMYND12	zinc finger MYND-type containing 12	Protein coding
ZNF837	zinc finger protein 837	Protein coding

Downregulated genes				
Gene	Name	Type of gene		
ALOX5AP	arachidonate 5-lipoxygenase activating protein	Protein coding		
ANTXR2	ANTXR cell adhesion molecule 2	Protein coding		
APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2	Protein coding		
ARHGAP10	Rho GTPase activating protein 10	Protein coding		
ARID5B	AT-rich interaction domain 5B	Protein coding		
ARL11	ADP ribosylation factor like GTPase 11	Protein coding		
C2orf74	chromosome 2 open reading frame 74	Protein coding		
CCDC7	coiled-coil domain containing 7	Protein coding		
CLDN15	claudin 15	Protein coding		
CLN3	CLN3 lysosomal/endosomal transmembrane protein, battenin	Protein coding		
COPS7A	COP9 signalosome subunit 7A	Protein coding		
CSPP1	centrosome and spindle pole associated protein 1	Protein coding		
DDIT3	DNA damage inducible transcript 3	Protein coding		
EIF4ENIF1	eukaryotic translation initiation factor 4E nuclear import factor 1	Protein coding		
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	Protein coding		
FYB	FYN-binding protein 1	Protein coding		
GBP5	guanylate binding protein 5	Protein coding		
HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	Protein coding		
KCTD6	potassium channel tetramerization domain containing 6	Protein coding		
KLHL20	kelch like family member 20	Protein coding		
LAIR1	leukocyte associated immunoglobulin like receptor 1	Protein coding		
LYRM4	LYR motif containing 4	Protein coding		
MIR155HG	MIR155 host gene	ncRNA		
NSUN4	NOP2/Sun RNA methyltransferase 4	Protein coding		
NUDT9	nudix hydrolase 9	Protein coding		
OSGEP	O-sialoglycoprotein endopeptidase	Protein coding		
PBX2	PBX homeobox 2	Protein coding		
PGBD2	piggyBac transposable element derived 2	Protein coding		
RRAGB	Ras related GTP binding B	Protein coding		
SDF2	stromal cell derived factor 2	Protein coding		
TATDN3	TatD DNase domain containing 3	Protein coding		
TCF4	transcription factor 4	Protein coding		
TM2D1	TM2 domain containing 1	Protein coding		
TMEM55B	transmembrane protein 55B	Protein coding		
TMEM69	transmembrane protein 69	Protein coding		
WRAP53	WD repeat containing antisense to TP53	Protein coding		
ZFPL1	zinc finger protein like 1	Protein coding		
ZNF616	zinc finger protein 616	Protein coding		

Supplementary Table 2. Top 10 pathways derived from ingenuity pathway analysis (IPA) from the ChIP-RNA integration. The 10 pathways were determined using IPA software according to p-value from Fisher's exact test.



Supplemental Figure S1. (A) K562 cells were treated for 24 hours with the indicated concentration of JQ1. Cells were then assessed for apoptosis using AnnexinV+ staining and flow cytometry at 24 hours post treatment; ns = not significant. (B) Colony forming unit assays were performed on two MLL wild type AML patients' samples, cells were plated in triplicates. Cells were treated with JQ1 at a concentration of 9 nM or 12.5 nM or with vehicle control (DMSO); ns = not significant, (C) Murine bone marrow cells from healthy mice were treated for 24 hours with the indicated concentration of JQ1. Cells were then assessed for apoptosis using AnnexinV+ staining and flow cytometry at 24 hours post treatment; ns = not significant. (D) Secondary transplants were performed and chimerism was analyzed as previously described (Dorrance AM et al. Leukemia. 2015;29(11):2143-2153). Percent chimerism (CD45.1 [healthy recipients] vs CD45.2 [MII^{PTD/WT} FIt3^{ITD/WT} leukemic donors]) was determined in the blood at defined time points after re-transplantation. (E) WST-1 assay on EOL-1 cells which were treated with a siRNA or a Scramble control as indicated for 48 hours. *P<0.05, ***P<0.001. (F) Normalized expression of human genes that were found to be downregulated after knockdown of MLL-PTD: ns = not significant, **P<0.01, ***P<0.001. (G) Cell lysate of EOL-1 cells transduced with either shScramble or shMLL-PTD were subjected to a chromatin immunoprecipitation (ChIP) assay. Antibodies against BRD4 or an unspecific IgG were used for the pulldown. DNA of ADAMDEC1 and SLAMF8 were quantified using qRT-PCR. The enriched binding of BRD4 to both genes, i.e. ADAMDEC1 and SLAMF8, was suppressed upon knockdown of MLL-PTD; *P<0.05. (H) Normalized expression of murine genes that were found to be downregulated after in vivo treatment in MIIPTD/WT FIt3ITD/WT leukemic mice. Adamdec1 and Slamf8 expressions were both decreased; ns = not significant, *P<0.05, **P<0.01, ***P<0.001. (I) Down regulation of Adamdec1 was validated in a Western Blot. (J) Humanized mouse model: all mice used in the experiments were between 8 and 10 weeks of age. EOL-1 mouse model: Female NOD-scid

IL2Rg^{null} (NSG) mice were transplanted with 1x10⁷ EOL-1 cells. Two weeks post-transplant mice were treated with JQ1 or DMSO vehicle control (6 mice per group; dosage 50 mg/kg body weight intraperitoneal), daily for 14 days. Bone marrow cells where isolated and expression of BRD4 downstream targets was determined. All studies using animals were carried out in accordance with the OSU institutional guidelines for animal care and under protocols approved by the OSU Institutional Animal Care and Use Committee.

