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

Marius Bill,[1,2] Chinmayee Goda,[1*] Felice Pepe,[1] Hatice Gulcin Ozer,[3] Betina McNeil,[1] Xiaoli Zhang,[3] Malith Karunasiri,[1] Rohan Kulkarni,[1] Sonu Kalyan,[1] Dimitrios Papaioannou,[1,4] Gregory Ferenchak,[1] Ramiro Garzon,[1,4] James E. Bradner,[5] Guido Marcucci,[6] Michael A. Caligiuri,[6] and Adrienne M. Dorrance[1,4]*

**MB and CG contributed equallly as co-first authors.*

[1]The Ohio State University, Comprehensive Cancer Center, Columbus, OH, USA; [2]Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Germany; [3]The Ohio State University, Department of Biomedical Informatics, Columbus, OH, USA; [4]Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA; [5]Dana-Faber Cancer Institute, Boston, MA, USA and [6]City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Correspondence: ADRIENNE DORRANCE - adrienne.dorrance@osumc.edu

doi:10.3324/haematol.2020.271627

Supplemental Data

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

Marius Bill[1,2*], Chinmayee Goda[1*], Felice Pepe[1], Hatice Gulcin Ozer[3], Betina McNeil[1], Xiaoli Zhang[3], Malith Karunasiri[1], Rohan Kulkarni[1], Sonu Kalyan[1], Dimitrios Papaioannou[1,4], Gregory Ferenchak[1], Ramiro Garzon[1,4], James E. Bradner[5], Guido Marcucci[6], Michael A. Caligiuri[6], and Adrienne M. Dorrance[1,4]

[1]The Ohio State University, Comprehensive Cancer Center, Columbus, OH, USA.
[2]University Hospital Dresden, Medical Clinic 1, Dresden, Germany
[3]The Ohio State University, Department of Biomedical Informatics, Columbus, OH, USA.
[4]Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA.
[5]Dana-Faber Cancer Institute, Boston, MA, USA.
[6]City of Hope Comprehensive Cancer Center, 1500 East Duarte Road, Duarte, CA 91010;
[*]These authors contributed equally to this work.

Correspondence: Adrienne Dorrance PhD, Division of Hematology, Department of Internal Medicine, The Ohio State University, 460 West 12th Avenue, Biomedical Research Tower 404, Columbus, 43210 OH, USA E-mail: adrienne.dorrance@osumc.edu

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.

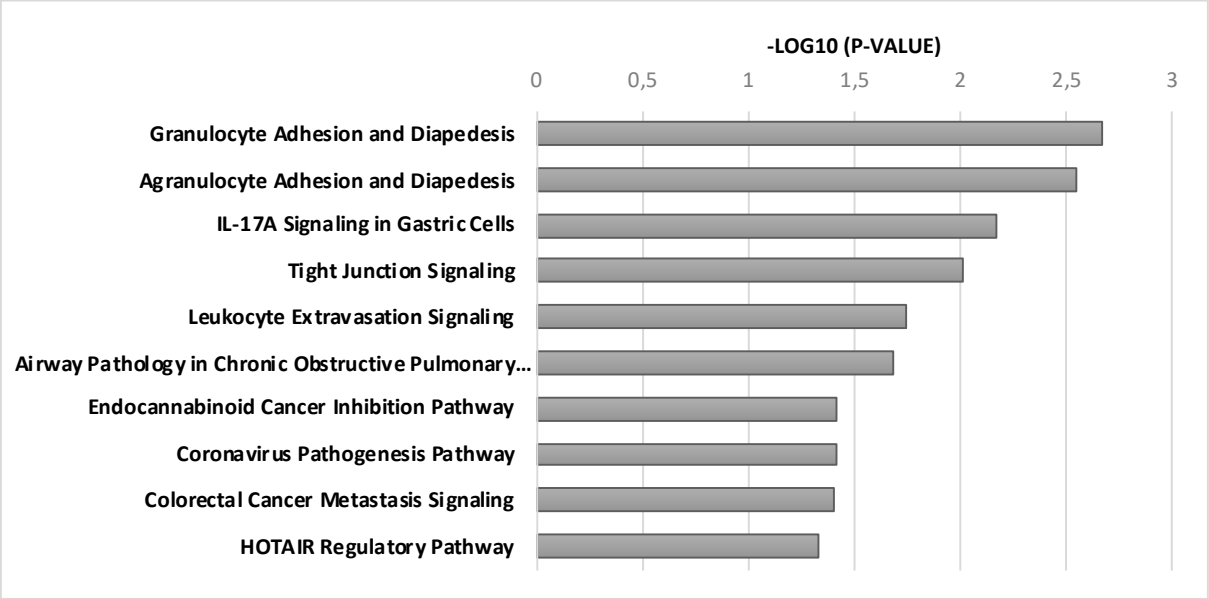
Upregulated genes		
Gene	**Name**	**Type of gene**
ACTR3C	actin related protein 3C	Protein coding
ADAMDEC1	ADAM like decysin 1	Protein coding
ANG	angiogenin	Protein coding
ASGR2	asialoglycoprotein receptor 2	Protein coding
AVPI1	arginine vasopressin induced 1	Protein coding
B3GNT8	UDP-GlcNAc:betaGal 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

FIZ1	FLT3 interacting zinc finger 1	Protein coding
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	Protein coding
GP1BA	glycoprotein Ib platelet subunit alpha	Protein coding
GRIN2D	glutamate ionotropic receptor NMDA type subunit 2D	Protein coding
ICAM5	intercellular adhesion molecule 5	Protein coding
IL18BP	interleukin 18 binding protein	Protein coding
IL21R	interleukin 21 receptor	Protein coding
IRF9	interferon regulatory factor 9	Protein coding
KCNK13	potassium two pore domain channel subfamily K member 13	Protein coding
KDM6B	lysine demethylase 6B	Protein coding
LMLN	leishmanolysin like peptidase	Protein coding
LOH12CR2	loss of heterozygosity, 12, chromosomal region 2	ncRNA
LONRF3	LON peptidase N-terminal domain and ring finger 3	Protein coding
LRRC25	leucine rich repeat containing 25	Protein coding
MICALL2	MICAL like 2	Protein coding
MIR143	microRNA 143	miRNA
MIR23A	microRNA 23a	miRNA
MIR3175	microRNA 3175	miRNA
MIR3178	microRNA 3178	miRNA
MIRLET7A3	microRNA let-7a-3	miRNA
MIRLET7B	microRNA let-7b	miRNA
MIRLET7I	microRNA let-7i	miRNA
MKRN3	makorin ring finger protein 3	Protein coding
MLNR	motilin receptor	Protein coding
MLPH	melanophilin	Protein coding
MMP2	matrix metallopeptidase 2	Protein coding
MSC	musculin	Protein coding
MTRNR2L2	MT-RNR2 like 2	Protein coding
NRTN	neurturin	Protein coding
ODF3	outer dense fiber of sperm tails 3	Protein coding
ODF3B	outer dense fiber of sperm tails 3B	Protein coding
OLIG1	oligodendrocyte transcription factor 1	Protein coding
PGAP2	post-GPI attachment to proteins 2	Protein coding
PGAP3	post-GPI attachment to proteins 3	Protein coding
PLEKHG3	pleckstrin homology and RhoGEF domain containing G3	Protein coding
PROM2	prominin 2	Protein coding
PRR3	proline rich 3	Protein coding
PTGER4	prostaglandin E receptor 4	Protein coding
QRFP	pyroglutamylated RFamide peptide	Protein coding
RDH5	retinol dehydrogenase 5	Protein coding
RHPN1	rhophilin Rho GTPase binding protein 1	Protein coding
RLN3	relaxin 3	Protein coding
S100A3	S100 calcium binding protein A3	Protein coding
S100P	S100 calcium binding protein P	Protein coding
SFTPB	surfactant protein B	Protein coding
SIT1	signaling threshold regulating transmembrane adaptor 1	Protein coding
SLAMF8	SLAM family member 8	Protein coding
SLC35B3	solute carrier family 35 member B3	Protein coding
SLC39A13	solute carrier family 39 member 13	Protein coding
SNAI3	snail family transcriptional repressor 3	Protein coding
SNAPC2	small nuclear RNA activating complex polypeptide 2	Protein coding
SNORD95	small nucleolar RNA, C/D box 95	snoRNA
SPDYC	speedy/RINGO cell cycle regulator family member C	Protein coding
TBX6	T-box 6	Protein coding
TNFSF12	TNF superfamily member 12	Protein coding
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

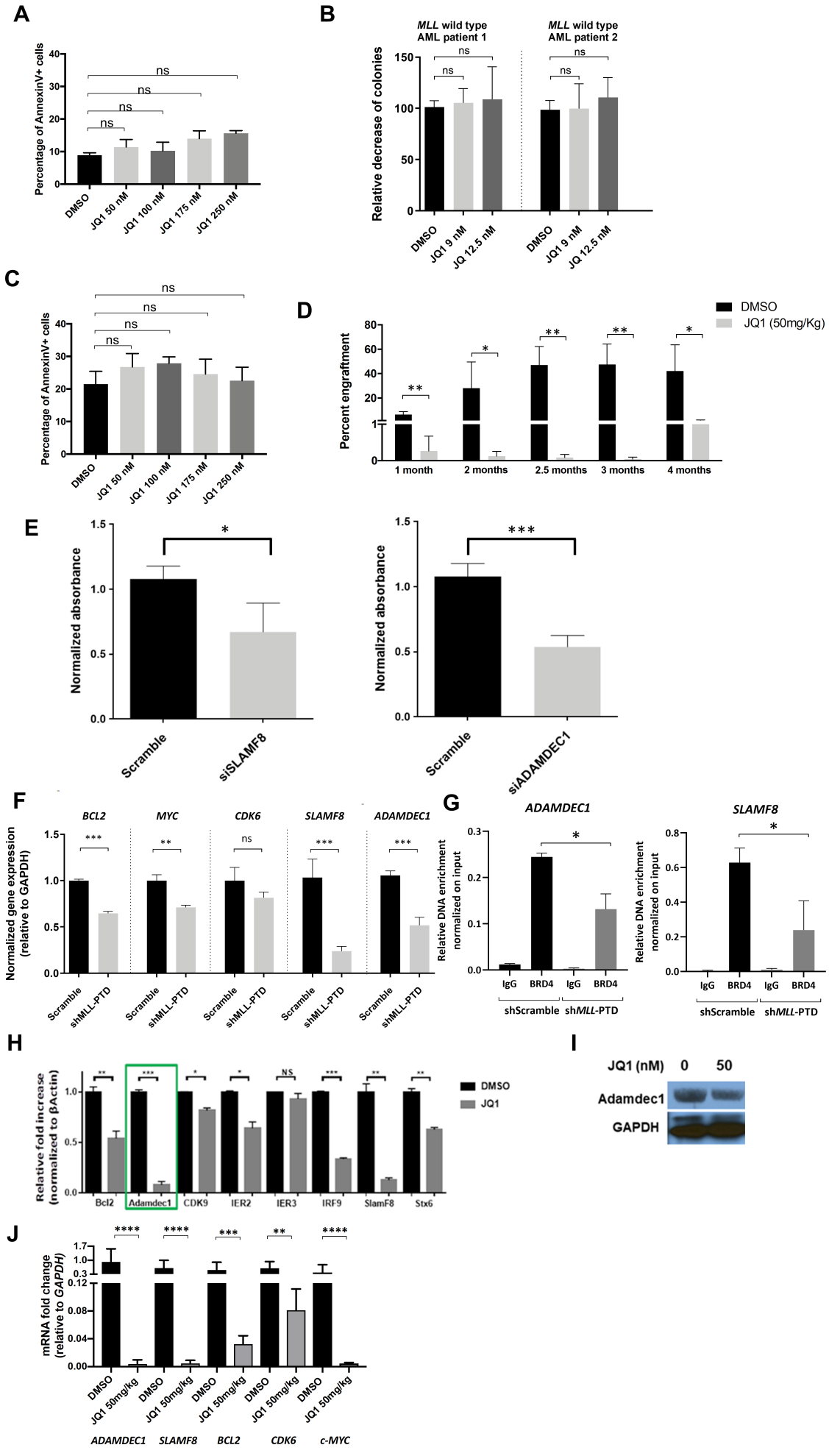
Downregulated genes		
Gene	**Name**	**Type of gene**
ALOX5AP	arachidonate 5-lipoxygenase activating protein	Protein coding
ANTXR2	ANTXR cell adhesion molecule 2	Protein coding
APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2	Protein coding
ARHGAP10	Rho GTPase activating 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 [*Mll*$^{PTD/WT}$ *Flt3*$^{ITD/WT}$ 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 sh*MLL*-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 *Mll*$^{PTD/WT}$ *Flt3*$^{ITD/WT}$ 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 1x10^{7} 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.


A
Percentage of AnnexinV+ cells
ns
DMSO
JQ1 50 nM
JQ1 100 nM
JQ1 175 nM
JQ1 250 nM
B
MLL wild type AML patient 1
MLL wild type AML patient 2
Relative decrease of colonies
DMSO
JQ1 9 nM
JQ 12.5 nM
C
Percentage of AnnexinV+ cells
D
DMSO
JQ1 (50mg/Kg)
Percent engraftment
1 month
2 months
2.5 months
3 months
4 months
E
Normalized absorbance
Scramble
siSLAMF8
siADAMDEC1
F
BCL2
MYC
CDK6
SLAMF8
ADAMDEC1
Normalized gene expression (relative to GAPDH)
Scramble
shMLL-PTD
G
ADAMDEC1
SLAMF8
Relative DNA enrichment normalized on input
IgG
BRD4
shScramble
shMLL-PTD
H
Relative fold increase (normalized to βActin)
DMSO
JQ1
Bcl2
Adamdec1
CDK9
IER2
IER3
IRF9
SlamF8
Stx6
I
JQ1 (nM) 0 50
Adamdec1
GAPDH
J
mRNA fold change (relative to GAPDH)
DMSO
JQ1 50mg/kg
ADAMDEC1
SLAMF8
BCL2
CDK6
c-MYC