Supplementary Methods

Western Blot

The membrane was then blocked in either 5% non-fat dry milk or 5% BSA followed by incubation with primary antibodies at 4°C overnight. Antibodies for p-STAT5 (Abcam 32364), STAT5 (Cell Signaling 9358), p-JAK2 (Cell Signaling 3774), JAK2 (Cell Signaling 3230), p-STAT3 (Cell Signaling 9145), STAT3 (Cell Signaling 9139), tubulin (Cell Signaling 2144), ac-tubulin (sigma T6793), H3 (Abcam 1791), ac-H3 (Millipore 06-599), p-AURKB (Cell Signaling 2914), AURKA (Cell Signaling 14475), p21Waf1/Cip1 (Cell Signaling 2947), p-CDK1 (Cell Signaling 9111), CDK1 (Abcam 32384), Cyclin B1 (Cell Signaling 12231), p27 (Cell Signaling 3686), p-H3 (Cell Signaling 3377), HDAC6 (Millipore 07-732) and HDAC11 antibody (NBP2-16789, Novus Biological) at a 1:1000 dilution in 5% bovine serum albumin in TBST buffer were used. Horseradish peroxidase-conjugated secondary antibodies were from Thermo Scientific.

Apoptosis analysis

Cells were washed twice with annexin V binding buffer (BD Bioscience 556454), and stained with annexin V-APC (BioLegend 640920) for 15 min in the dark. DAPI (Invitrogen D3571) was added to stain dead cells before acquisition by a LSRII cytometer (BD Biosciences). Analysis was performed using FlowJo software version10.0.8 (Tree Star).

siRNA Silencing

siRNA transfection was performed using Lonza Cell Line Nucleofector Kit V (Lonza

VCA-1003) on Amaxa Nucleofector 2b (Lonza) according to the manufacturer's instructions. HDAC6 siRNAs were purchased from Dharmacon (J-003499-05-0005 for siRNA#1 and J-003499-06-0005 for siRNA#2), as was the non-targeting control siRNA (Dharmacon D-001810-01-05).

Preparation of lentivirus and HDAC11 knock down

HEK293 cell line was used to obtain lentivirus from packaging plasmids and Mission HDAC11 shRNA vector (Clone ID: NM_024827.3-364s21c1). Sequence: CCGGG CGCTA TCTTA ATGAG CTCAA CTCGA GTTGA GCTCA TTAAG ATAGC GCTTT TTG. Also, a Non-Target shRNA was used as a control plasmid. 4×10^6 HEK 293 cells were seeded into 10 cm tissue culture plates in 10 ml of growth medium a day before transfection. Next day, 70-80% confluent cells were transfected with prescribed DNA concentrations using jet prime transfection reagent (POLYPLUS, Cat# 114-07) according to manufacturer's protocol and placed in 37°C degree CO_2 incubator. Fresh antibiotics free medium was added into transfected plates after 24 hours and incubated for additional 24 hours. Lentiviral supernatants were passed through a 0.45 μm low protein binding filter to remove cellular debris 48 hours after the start of transfection when the viral titers were generally highest. The shRNA lentiviruses were concentrated by ultracentrifugation (2 hours at 23,000 rpm) and re-suspended in 100uL of serum free media.

Total 0.5×10^6/100uL of *JAK2*^{V617F}-expressing Ba/F3 cells were spinfected for 2 hours (1500 rpm) with polybrene and 20uL of virus titer in a 96 well plate. After spinfection cells

were diluted into 1mL of growth media and monitored for metabolic health every 24 hours post transduction using cell titer glow assay (CellTiter-Glo® Luminescent Cell Viability Assay, Promega, Cat# G7570) according to the manufacturer's protocol.

50 μL of cells equilibrated at room temperature (control non-target (NT) shRNA and HDAC11 shRNA lentivirus transduced) were plated in a clear bottom black 96-multi well plate and mixed with an equal volume of cell titer glow mix (prepared by mixing cell titer glow buffer and lyophilized substrate according to manufacturer's instructions). Cell mix was lysed on a plate shaker for 2 minutes and incubated for another 10 minutes in the dark. The luminescence signal as a measure of intracellular ATP levels was recorded using standard luminometer.

A second constitutively expressed HDAC11 shRNA construct (TRCN0000330863) was also used in some experiments as indicated (SET-2 cells shown in Supplementary Fig. S3i) and JAK2 mutated Ba/F3 cells (Supplementary Fig. S3g-h). The transient knock down of HDAC11 expression is measured after 72- and 96-hours post lentivirus infection.

In SET2 cells, HDAC11 shRNA knockdown was performed using IPTG inducible shRNA vectors MISSION TRC2 pLKO.5-IPTG-3xLAc0 (TRCN0000199149 & TRCN0000330863) and compared to the control MISSSION TRC2 pLKO.5-puro-IPTG-3xLAc0 (SHC202) (Sigma Aldrich, St. Louis, MO). Lentiviral particles were produced in HEK-293T cells with jetPRIME transfection reagent (Polyplus, Illkirch, France) and 3rd generation lentiviral packaging mixture (Applied Biological Materials Inc., Richmond, Canada) according to the

manufacturer's protocol. Transduction was performed at 5 × 10^7 cells/mL for 2 h at 1500xg, room temperature (RT) in the presence of 0.6 μg/ml polybrene (Merck Millipore, Billerica, MA). The shRNA lentivirus was concentrated by ultracentrifugation (2 hours at 23,000 rpm) and re-suspended in 100uL of serum free media. Total 5x10^5/100uL of cells were spinfected for 2 hours (1500 rpm) with polybrene and 20uL of virus in a 96 well plate. Selection of stable clones started 48 hours post transduction by the addition of 1-2 μg/ml puromycin for 15-21 days. The stable shRNA expressing clones are induced with 300 μM IPTG and total HDAC11 expression is measured after 72- or 96-hours post IPTG induction. In this assay, Celigo imaging was used to assess viability. Celigo Imaging Cytometer compatible clear bottom black 96 well plates were used for imaging cells (Greiner-655090) according to the manufacturer's instructions (Nexcelom). Cell numbers and viability measurements were taken by staining 50 μL of cells with equal volumes of Acridine orange and Propidium Iodide (AOPI, Thermofisher, CS2-0106) solution in a 96 well plate as described in the vendor protocol. The stained cells are incubated for not more than 10 minutes and imaged in green (Acridine orange) and red (propidium iodide) channels to measure live and dead cells, respectively.

Cell cycle analyses

For cell cycle analysis, cells were first fixed in 70% ethanol overnight. The next day, cells were washed with PBS and suspended in DAPI/Triton X-100 staining solution (0.1% Triton X-100, 1ug/ml DAPI) and kept in dark for 1 hr before measured by a LSRII cytometer (BD

Biosciences,). Analysis was performed using ModFit LT software v4.1 (Verify Software House).

Mouse bone marrow colony assays

0.2 million mouse BM cells were suspended in 200-µl RPMI, which was added to 3-ml Methocult medium (Stem Cell Technologies 03444) with or without cytokines (Stem Cell Technologies 03231) and vortexed for 15 sec. When bubbles disappeared 10 min later, Methocult medium was dispensed into culture dishes and incubated at 37°C and 5% CO_2. Colonies were observed and counted after incubation for 7 days. Colonies were stained by 1mg/ml MTT overnight to take representative pictures.

Human bone marrow colony assays

Colony formation of primary human MPN cells was performed as described previously [1]. Briefly, mononuclear cells were isolated from peripheral blood obtained from Moffitt Cancer Center patients who were consented through the Moffitt Cancer Center Total Cancer Care protocol (MCC 14690/Liberty IRB #12.11.0023), with approval by the Moffitt Cancer Center Scientific Review committee. Colony formation was assessed by plating 0.2 million cells in duplicate in methylcellulose lacking erythropoietin (MethoCult™ #H4534; STEMCELL Technologies, Inc.). Primary samples were from patients with ET, PV or MF as indicated (Fig. 3C-D). All patients were *JAK2*^{V617F} positive and none of the patients were on ruxolitinib at the time of sample acquisition.

qRT-PCR analysis

Total RNA was extracted using RNeasy Micro Kit (Qiagen), and converted into cDNA using iScript cDNA synthesis kit (Bio-Rad Laboratories) according to the manufacturer's instructions. cDNA was added to Taqman PCR mix (Life Technologies) in a 25-μL final volume containing forward and reverse primers. Amplification cycles (n=40) were performed using 7900HT Real-time PCR System (Applied Biosystems). Primers were purchased from Life Technologies, Carlsbad, CA (*CDKN1A* Hs00427201_CE), TATA-binding protein (*TBP*) Mm00446971_m1). Data were normalized to the reference gene *TBP* by a relative quantification using the ΔΔCt method.

Analysis by LC-MS/MS Mass spectrometry

The gel regions between 75 and 100 kDa for HSP90 immunoprecipitates from cells with HDAC6 siRNA knockdown and NTC siRNA were excised and treated with Tris(2-carboxy-ethyl) phosphine hydrochloride (TCEP) and iodoacetamide to reduce disulfide bones and alkylate resulting from free cysteines. In-gel digestion using trypsin was performed overnight at 37 ºC. After extraction and vacuum concentration, the tryptic peptides were analyzed using nanoflow liquid chromatography (RSLCnano, Thermo, San Jose, CA) coupled to an electrospray hybrid quadrupole Orbitrap mass spectrometer (Q Exactive Plus, Thermo, San Jose, CA) in a data-dependent manner for tandem mass spectrometry peptide sequencing. Peptides were first loaded onto a pre-column (5mm x 300 μm ID packed with C18 reversed-phase resin, 5μm particle size, 100Å pore size) and washed for 8 minutes with aqueous 2% acetonitrile and 0.04% trifluoroacetic acid. The

trapped peptides were eluted onto a 75 μm ID × 25 cm, 2 μm Particle size, 100Å pore size, C18 analytical column (Dionex, Sunnyvale, CA) using a 90-minute program at a flow rate of 300 nL/min of 5% solvent B for 8 minutes, 5 to 38.5% solvent B over 60 minutes, then 50% to 90% solvent B over 7 minutes and held at 90% for 5 minutes, followed by 90% to 5% solvent B in 1 minute and re-equilibrate for 10 minutes. Solvent A was composed of 98% ddH_2O and 2% acetonitrile containing 0.1% FA. Solvent B was 90% acetonitrile and 10% ddH_2O containing 0.1% FA. Sixteen tandem mass spectra were collected in a data-dependent manner following each survey scan.The relative quantification of peptides was calculated using MaxQuant (version 1.2.2.5). Peaks were searched against the Human database (20,151 sequences, released August 2015) with the Andromeda search engine[2]. The raw files were processed using select parameters including at least seven amino acids per peptide, as many as three missed cleavages to accommodate lysine acetylation sites, and a false discovery rate of 0.01 was selected for peptides and proteins. Methionine oxidation and peptide lysine and N-terminal acetylation were selected as variable modifications in protein quantification. Skyline version 3.7 was used for data evaluation using extracted ion chromatograms for HSP90 peptides[3].

Gene set enrichment analysis for differentially-expressed genes

For gene set enrichment analysis (GSEA), the GSEA java based desktop software was utilized in conjunction with a pre-ranked list of differentially expressed genes (2-fold change, FDR<0.05) between FT234 and control treated cells at 12 hours. Enrichment was

tested for in the C3 transcription factor collection and in the hallmark collection from the MSigDB (Broad Institute).

Table S1. Flow cytometry instrument settings

Laser			Detector	Optical Filters		Fluorochrome
Wavelength (nm)	Power (nm)	Type	Spectral Range (nm)	Long Pass (nm)	Band Pass (nm)	
488	50	DPSS	685-735	685	710/50	PerCP-Cy5.5
			505-525	505	515/20	FITC, BB515
			483-493	--	488/10	SSC
405	100	DPSS	755-810	755	780/60	BV785
			685-735	670	710/50	BV711
			650-670	630	660/20	BV650
			600-620	590	610/20	BV605
			570-607*	570	585/42	BV570
			505-525	505	515/20	BV510
			425-475	--	450/50	BV421
640	100	DPSS	750-810	740	780/60	APC-H7/Cy7
			685-735	685	710/50	AF700
			663-677	--	670/14	AF647, APC
532	150	DPSS	760-800	740	780/40	PE-Cy7
			690-735*	690	710/50	PE-Cy5.5
			640-680	640	660/40	PE-Cy5
			600-620	595	610/20	PE-CF594
			562-588	--	575/26	PE

355	20	DPSS	790-850	770	820/60	BUV805
			500-530	450	515/30	BUV496
			365-393	--	379/28	BUV395

Instrument Configuration. This panel was developed and optimized for use on BDLSRII flow cytometry instrument with the listed optical configuration.

Table S2. Flow cytometry antibodies and experimental design

Antibody						Staining Conditions			
Specificity	Fluorochrome	Clone	Vendor	Catalog Number	Vol/Test (μL)	Vol (μL)	T (C)	t (min)	Step
Mature Populations									
CD45	PE/CF594	30-F11	BD	562420	1	100	4	60	EC
CD3	BV711 or FITC*	145-2C11	BL BD	100349 553061	1	100	4	60	EC
B220	PerCP/Cy5.5	RA3-6B2	BL	103236	1	100	4	60	EC
CD41	BV605	MWReg30	BL	133921	1	100	4	60	EC
F4/80	BUV395	T45-2342	BD	565614	1	100	4	60	EC
CD11b	APC	M1/70	BL	101212	1	100	4	60	EC
Ter119	BV510	TER-119	BL	116237	1	100	4	60	EC
CD71	PE/Cy7	RI7217	BL	113812	1	100	4	60	EC
DAPI			TF	D3571	1	100	4	60	EC
Lineage Cocktail									
CD3	Biotin	145-2C11	BL	100304	1	100	4	60	EC
CD4	Biotin	RM4-5	eBio	13-0042-85	1	100	4	60	EC
CD8	Biotin	53-6.7	BL	100704	1	100	4	60	EC
CD11b	Biotin	M1/70	BD	553309	1	100	4	60	EC
CD19	Biotin	6D5	BL	115504	1	100	4	60	EC

CD127	Biotin	B12-1	BD	555288	1	100	4	60	EC
B220	Biotin	RA3-6B2	BL	103204	1	100	4	60	EC
Gr-1	Biotin	RB6-8C5	BD	553125	1	100	4	60	EC
Ter119	Biotin	TER-119	BL	116204	1	100	4	60	EC
Stem and Progenitor Populations									
CD45	PE/CF594	30-F11	BD	562420		100	4	60	EC
Streptavidin	APC-Cy7		BL	405208	1	100	4	60	EC
c-Kit	APC	2B8	BL	105812	1	100	4	60	EC
Sca-1	PE-Cy7	D7	BL	108114	1	100	4	60	EC
CD34	PE	RAM34	BD	551387	4	100	4	60	EC
CD135	BV421	A2F10	BL	135314	1	100	4	60	EC
CD150	PerCP/Cy5.5	TC15-12F 12.2	BL	115922	1	100	4	60	EC
CD 48	BV 605	HM48-1	BL	103441	1	100	4	60	EC
Ghost Dye Viability Dye	BV510		Tonbo	TB-13-087 0-T500	1	100	4	60	EC
Congenic Markers									
CD45.1	PE	A20	Tonbo	TB-50-045 3-U100	1	100	4	60	EC
CD45.2	APC/Cy7	104	Tonbo	TB-25-045 4-U100	1	100	4	60	EC

Commercial reagents used in the final panel (Abbreviations: BL, BioLegend; BD, Becton-Dickinson; eBio, Affymetrix eBioscience; Tonbo, Tonbo Biosciences; TF, Thermo Fischer Scientific; EC, extracellular staining. *CD3 BV711 was used with samples that expressed GFP.

Table S3. IC_{50} values for compounds tested in specific cell lines.

IC_{50} (μM)					
	MPL^{W515L} Ba/F3	$JAK2^{V617F}$ Ba/F3	HEL92.1.7	SET-2	Figure
Compound name					
Ruxolitinib	0.258	0.053	1.062	0.29	Fig. S1a
Panobinostat (LBH589)	0.007	0.02	0.003	0.0024	Fig. S1b
Entinostat (MS275)	0.54	1.3	0.47	2.043	Fig. S1e
Nexturastat	0.2886	0.1114	0.2032	0.38	Fig. 1a
FT108	n/a	n/a	n/a	n/a	Fig. 1c
FT234	3.73	2.341	3.08	6.416	Fig. 2a-d
FT743	9.235	3.165	8.322	12.41	Fig. 2a-d
FT895	6.47	3.002	4.94	9.32	Fig. 2a-d
FT383	5.979	3.947	5.34	16.34	Fig. 2a-d
FT650	n/a	n/a	n/a	n/a	Fig. 2a-d
FT422	n/a	n/a	n/a	n/a	Fig. 2a-d

n/a=not applicable

Table S4. Pathway analyses (supplement for Figure 4)

TRANSCRIPTION FACTORS

Positive Enrichment

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
V$CREB_Q2	25	0.66688585	2.3965702	0	0.0033716	0.003	57	tags=40%, list=9%, signal=42%
V$ATF3_Q6	17	0.71155024	2.3356655	0	0.0016858	0.003	61	tags=47%, list=10%, signal=51%
V$CREB_Q4	23	0.6386501	2.277921	0	0.0038223	0.009	57	tags=35%, list=9%, signal=37%
V$CEBPDELTA_Q6	16	0.6797257	2.2102153	0	0.0062984	0.02	61	tags=38%, list=10%, signal=41%
YTATTTTNR_V$MEF2_02	33	0.5606475	2.165139	0	0.0099945	0.041	62	tags=36%, list=10%, signal=38%
V$CREB_Q2_01	17	0.6598964	2.147131	0.0012077	0.0100767	0.05	53	tags=41%, list=9%, signal=44%
TTANTCA_UNKNOWN	32	0.5475376	2.1071916	0	0.0152895	0.086	123	tags=56%, list=20%, signal=67%
V$ATF_01	21	0.6115113	2.0984845	0	0.0143876	0.092	57	tags=33%, list=9%, signal=36%
V$CREL_01	17	0.62338	2.0770612	0	0.0157852	0.115	171	tags=76%, list=28%, signal=104%
V$NFKAPPAB65_01	16	0.64712954	2.045359	0.0037129	0.0205368	0.165	171	tags=81%, list=28%, signal=110%
V$CREB_01	24	0.56775814	2.0375197	0.0023613	0.0202501	0.179	42	tags=25%, list=7%, signal=26%
TGACCTY_V$ERR1_Q2	37	0.5199274	2.031554	0.0010977	0.0191442	0.183	58	tags=27%, list=10%, signal=28%
V$CREBP1_Q2	18	0.5917336	2.010612	0.0036496	0.0209967	0.215	57	tags=39%, list=9%, signal=42%
CTAWWWATA_V$RSRFC4_Q2	16	0.617348	2.000791	0.0063211	0.0214261	0.233	1	tags=13%, list=0%, signal=12%
V$FREAC2_01	23	0.5522993	1.9536813	0.0034404	0.0309481	0.328	123	tags=57%, list=20%, signal=68%
V$NFKB_Q6_01	15	0.6065788	1.9445336	0.003736	0.0308571	0.342	228	tags=93%, list=38%, signal=146%
GGATTA_V$PITX2_Q2	20	0.5627635	1.9167831	0.0083933	0.0357364	0.404	118	tags=60%, list=20%, signal=72%
WGTTNNNNNAAA_UNKNOWN	27	0.5162693	1.8969411	0.0011641	0.0406076	0.454	70	tags=41%, list=12%, signal=44%
YYCATTCAWW_UNKNOWN	15	0.5922827	1.8750039	0.0075758	0.046466	0.513	42	tags=27%, list=7%, signal=28%
V$TATA_C	16	0.5757459	1.8627973	0.0103896	0.0483233	0.544	66	tags=31%, list=11%, signal=34%
RGAANNTTC_V$HSF1_01	27	0.50229573	1.8572506	0.0113636	0.0485992	0.558	70	tags=30%, list=12%, signal=32%
TGANTCA_V$AP1_C	53	0.4324205	1.8484384	0.007384	0.0499901	0.588	116	tags=40%, list=19%, signal=45%
GTGACGY_V$E4F1_Q6	38	0.46265855	1.8313535	0.0097508	0.0531402	0.624	125	tags=39%, list=21%, signal=47%
TGAYRTCA_V$ATF3_Q6	32	0.45983467	1.7822039	0.0134831	0.0733194	0.772	24	tags=16%, list=4%, signal=15%

Negative Enrichment

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
GCCATNTTG_V$YY1_Q6	15	-0.2050847	-0.9369059	0.519802	0.5295463	0.999	485	tags=100%, list=80%, signal=492%

HALLMARKS

Positive Enrichment

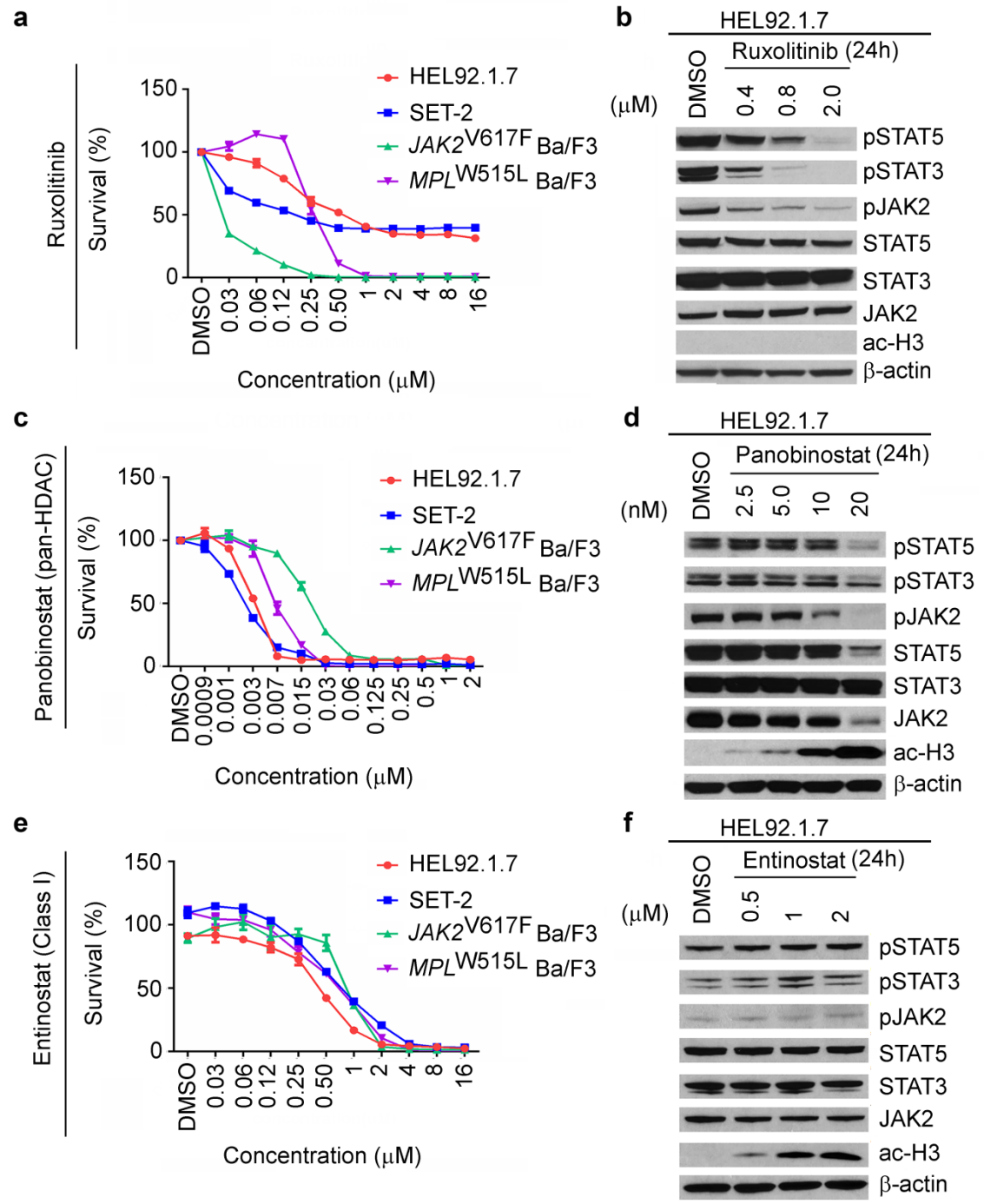
NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_TNFA_SIGNALING_VI	55	0.7161799	3.0556304	0	0	0	64	tags=51%, list=11%, signal=52%
HALLMARK_HYPOXIA	38	0.66722083	2.6609375	0	0	0	74	tags=50%, list=12%, signal=53%
HALLMARK_APOPTOSIS	22	0.7685602	2.6260316	0	0	0	38	tags=50%, list=6%, signal=51%
HALLMARK_P53_PATHWAY	21	0.6956952	2.4532094	0	0	0	74	tags=52%, list=12%, signal=58%
HALLMARK_EPITHELIAL_MESEN(	16	0.7317256	2.39576	0	0	0	102	tags=69%, list=17%, signal=81%
HALLMARK_IL2_STAT5_SIGNALIN	19	0.65740263	2.1897368	0	0.0011274	0.007	74	tags=53%, list=12%, signal=58%
HALLMARK_UV_RESPONSE_UP	20	0.6002329	1.9897634	0.001182	0.0048313	0.036	57	tags=35%, list=9%, signal=37%
HALLMARK_INFLAMMATORY_RES	22	0.5392168	1.8448006	0.0094229	0.0157697	0.13	89	tags=45%, list=15%, signal=51%
HALLMARK_KRAS_SIGNALING_U	17	0.48430178	1.5754782	0.0702179	0.0756174	0.541	131	tags=41%, list=22%, signal=51%
HALLMARK_MTORC1_SIGNALING	22	0.38139647	1.3793769	0.1408284	0.1642551	0.845	106	tags=36%, list=18%, signal=42%
HALLMARK_HEME_METABOLISM	17	0.41349268	1.3533674	0.1766201	0.163384	0.875	271	tags=76%, list=45%, signal=135%

Negative Enrichment

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_G2M_CHECKPOINT	29	-0.6810808	-4.0975122	0	0	0	111	tags=83%, list=18%, signal=96%
HALLMARK_E2F_TARGETS	23	-0.6219793	-3.490275	0	0	0	117	tags=83%, list=19%, signal=99%
HALLMARK_GLYCOLYSIS	17	-0.2479247	-1.2044097	0.2	0.2139199	0.37	35	tags=29%, list=6%, signal=30%

SUPPLEMENTARY FIGURES

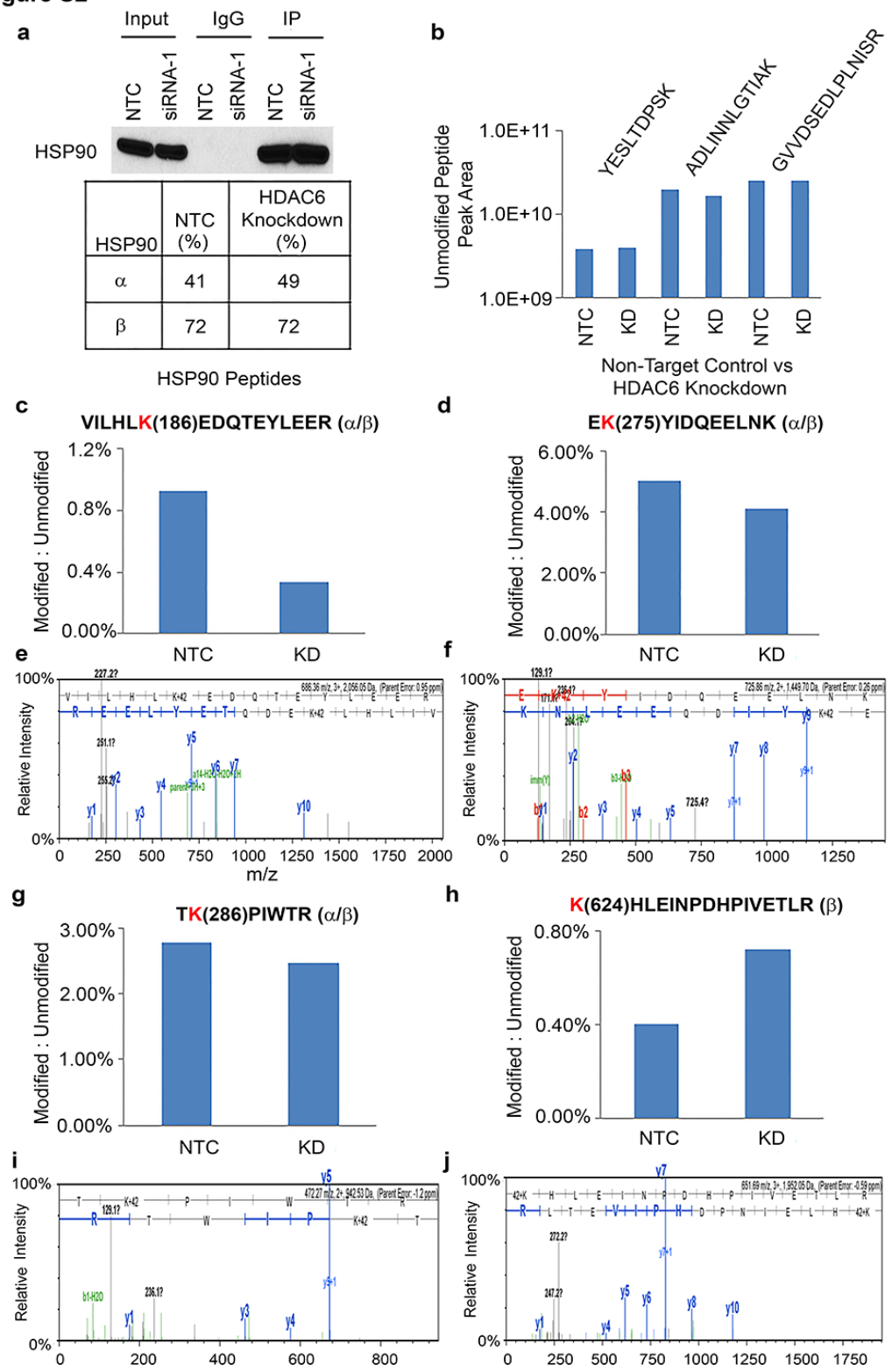
Figure S1



Supplementary Fig. S1 (Relevant to data shown in Fig. 1). Pan-HDAC inhibitors and class I HDAC

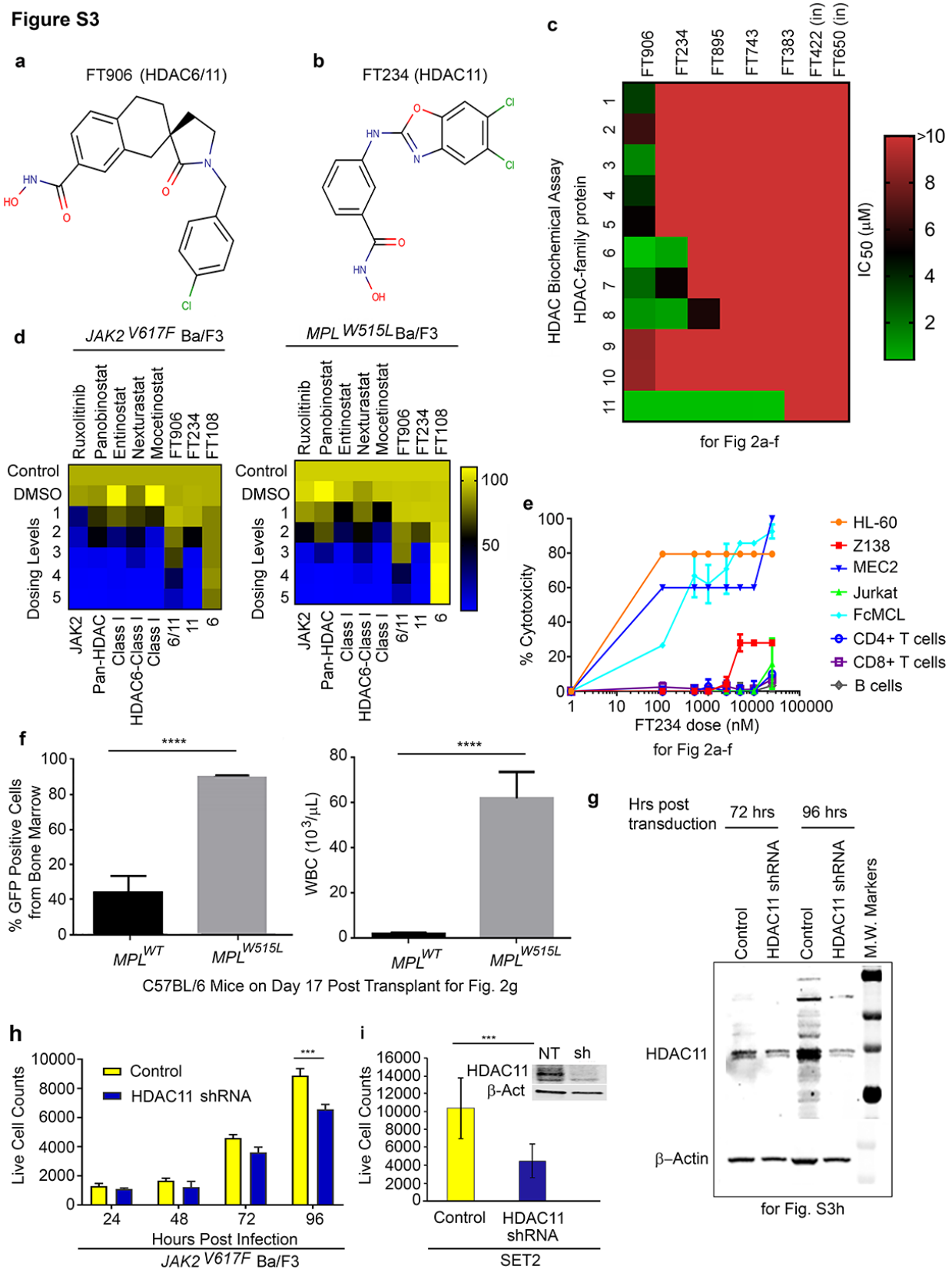
inhibitors suppress proliferation of human- and mouse MPN cell lines. (**a-f)**, HEL92.1.7 and SET-2 (human) as well as *JAK2*^{V617F} and *MPL*^{W515L}-transduced mouse Ba/F3 cells were treated with increasing doses of Ruxolitinib (**a-b**), Panobinostat (**c-d**), or Entinostat (**e-f**) for 48 hrs. Cell survival was measured by CCK8 assay and western blot analysis of the indicated signaling proteins was determined after 24 hrs of treatment in HEL92.1.7 cells. Ac=acetylated proteins; pSTAT5, pSTAT3, pJAK2= phosphorylated proteins. IC_{50} data provided in Table S3.

Figure S2


a
Input
IgG
IP
NTC
siRNA-1
NTC
siRNA-1
NTC
siRNA-1
HSP90
HSP90
NTC (%)
HDAC6 Knockdown (%)
α
41
49
β
72
72
HSP90 Peptides
b
Unmodified Peptide Peak Area
1.0E+11
1.0E+10
1.0E+09
YESLTDPSK
ADLINNLGTIAK
GVVDSEDLPLNISR
NTC
KD
NTC
KD
NTC
KD
Non-Target Control vs HDAC6 Knockdown
c
VILHLK(186)EDQTEYLEER (α/β)
Modified : Unmodified
1.2%
0.8%
0.4%
0.00%
NTC
KD
d
EK(275)YIDQEELNK (α/β)
Modified : Unmodified
6.00%
4.00%
2.00%
0.00%
NTC
KD
e
Relative Intensity
100%
0%
0
250
500
750
1000
1250
1500
1750
2000
m/z
f
Relative Intensity
100%
0%
0
250
500
750
1000
1250
g
TK(286)PIWTR (α/β)
Modified : Unmodified
3.00%
2.00%
1.00%
0.00%
NTC
KD
h
K(624)HLEINPDHPIVETLR (β)
Modified : Unmodified
0.80%
0.40%
0.00%
NTC
KD
i
Relative Intensity
100%
0%
0
200
400
600
800
j
Relative Intensity
100%
0%
0
250
500
750
1000
1250
1500
1750

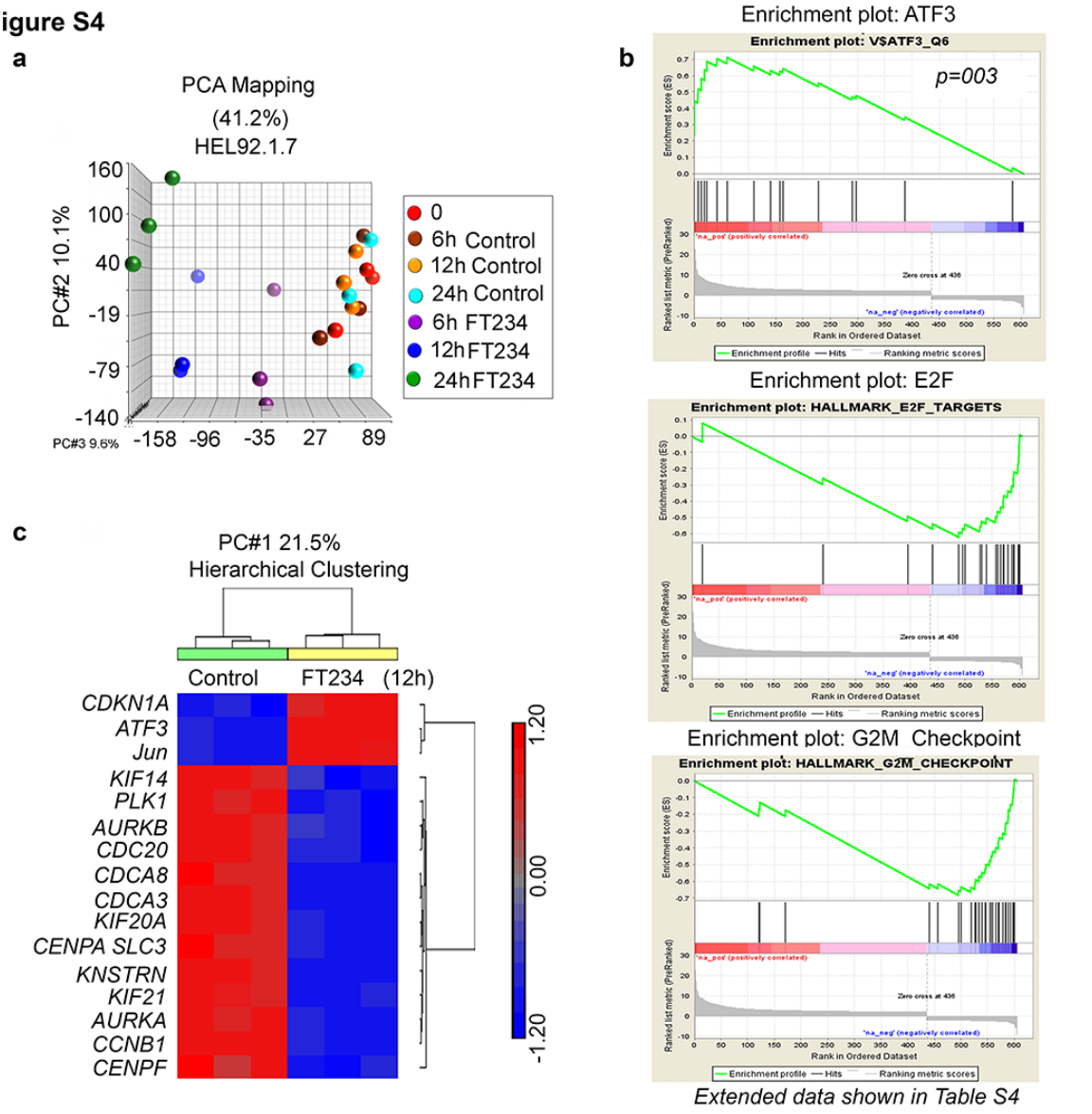
Supplementary Fig. S2 LC-MS/MS Analysis of HSP90 Acetylation in Control and HDAC6 Knockdown Cells. HSP90 was immunoprecipitated from HEL cells treated with non-targeted control (NTC) siRNA and HDAC6 siRNA. **(a)** The immunoprecipitated lysates were blotted against α-HSP90 antibody then subjected to liquid chromatography/tandem mass spectrometry. Sequence coverage (%) of HSP 90-α and β in NTC and HDAC6 knockdown (KD) samples are shown. **(b)** Peak area for three unmodified peptides identified in both HSP90-α and β from NTC and HDAC6 knockdown. No significant changes were observed in the unmodified peptides between NTC and HDAC6 KD, therefore the changes observed in the acetyl modified peptides are significant. **(c, d and g)** Percentage of acetyl modified to unmodified peptides identified in HSP 90-α and β and (h) an acetyl modified peptide identified in only HSP 90-β. **(e, f, i and j)** Spectra of acetyl modified peptides from HSP 90-α and β in NTC and HDAC6 knockdown samples. Peptides shown in **(e, f and i)** were identified in both HSP 90-α and β and shown in **(j)** was identified in only HSP90-β.

Figure S3


a
FT906 (HDAC6/11)
b
FT234 (HDAC11)
c
FT906
FT234
FT895
FT743
FT383
FT422 (in)
FT650 (in)
HDAC Biochemical Assay
HDAC-family protein
IC 50 (μM)
>10
8
6
4
2
for Fig 2a-f
d
JAK2 V617F Ba/F3
MPL W515L Ba/F3
Ruxolitinib
Panobinostat
Entinostat
Nexturastat
Mocetinostat
FT906
FT234
FT108
Control
DMSO
Dosing Levels
JAK2
Pan-HDAC
Class I
HDAC6-Class I
6/11
11
6
100
50
e
% Cytotoxicity
FT234 dose (nM)
HL-60
Z138
MEC2
Jurkat
FcMCL
CD4+ T cells
CD8+ T cells
B cells
for Fig 2a-f
f
% GFP Positive Cells from Bone Marrow
WBC (10^3/μL)
MPL^WT
MPL^W515L
****
C57BL/6 Mice on Day 17 Post Transplant for Fig. 2g
g
Hrs post transduction
72 hrs
96 hrs
Control
HDAC11 shRNA
M.W. Markers
HDAC11
β–Actin
for Fig. S3h
h
Live Cell Counts
Control
HDAC11 shRNA
***
Hours Post Infection
JAK2 V617F Ba/F3
i
NT
sh
HDAC11
β-Act
SET2

Supplementary Fig. S3 (Relevant to data shown in Fig. 2). HDAC inhibitors show differential activities in MPN cell lines. **(a)** Structure of FT906 and, **(b)** FT234. (**c**) IC_{50} concentrations of the HDAC inhibitors in suppressing each of the indicated HDACs were determined by an enzymatic assay. Color bar indicates doses ranging from 0 μM (green) to >10 μM (red). (**d**) *JAK2*^{V617F} and *MPL*^{W515L} Ba/F3 cells treated with the indicated HDAC inhibitors at five increasing doses for 48 hr, after which CCK8 assay were conducted to assess survival. Color bar indicates survival rate ranging from 0 (blue) to 100% (yellow) relative to DMSO. **(e)** % cytotoxicity of several cell lines as named along with purified human T cells and B cells. IC_{50}s for these experiments were not valid after calculation but showed distinct differences in sensitivity in at least three independent experiments. **(f)** % GFP positive BM cells and WBC on day 17 post-transplant with *MPL*^{WT} or *MPL*^{W515L}-transduced bone marrow. Experiment provided as supporting data for Fig. 2g. **(g)** Western blot analysis for HDAC11 and β-actin for cells treated with Non-target control (Control) or HDAC11 shRNA 72 or 96 hours after viral transduction. Cellular Viability using Cell titre glo as well as Acridine orange is measured in (**h**) mouse Ba/F3 *JAK2*^{V617F} and (**i**) human SET2 cells. The achieved impact on HDAC11 protein expression with transient HDAC11 knock down in SET2 cells. Statistical analysis performed using two-tailed unpaired t-test. **** $p < 0.0001$.

Figure S4

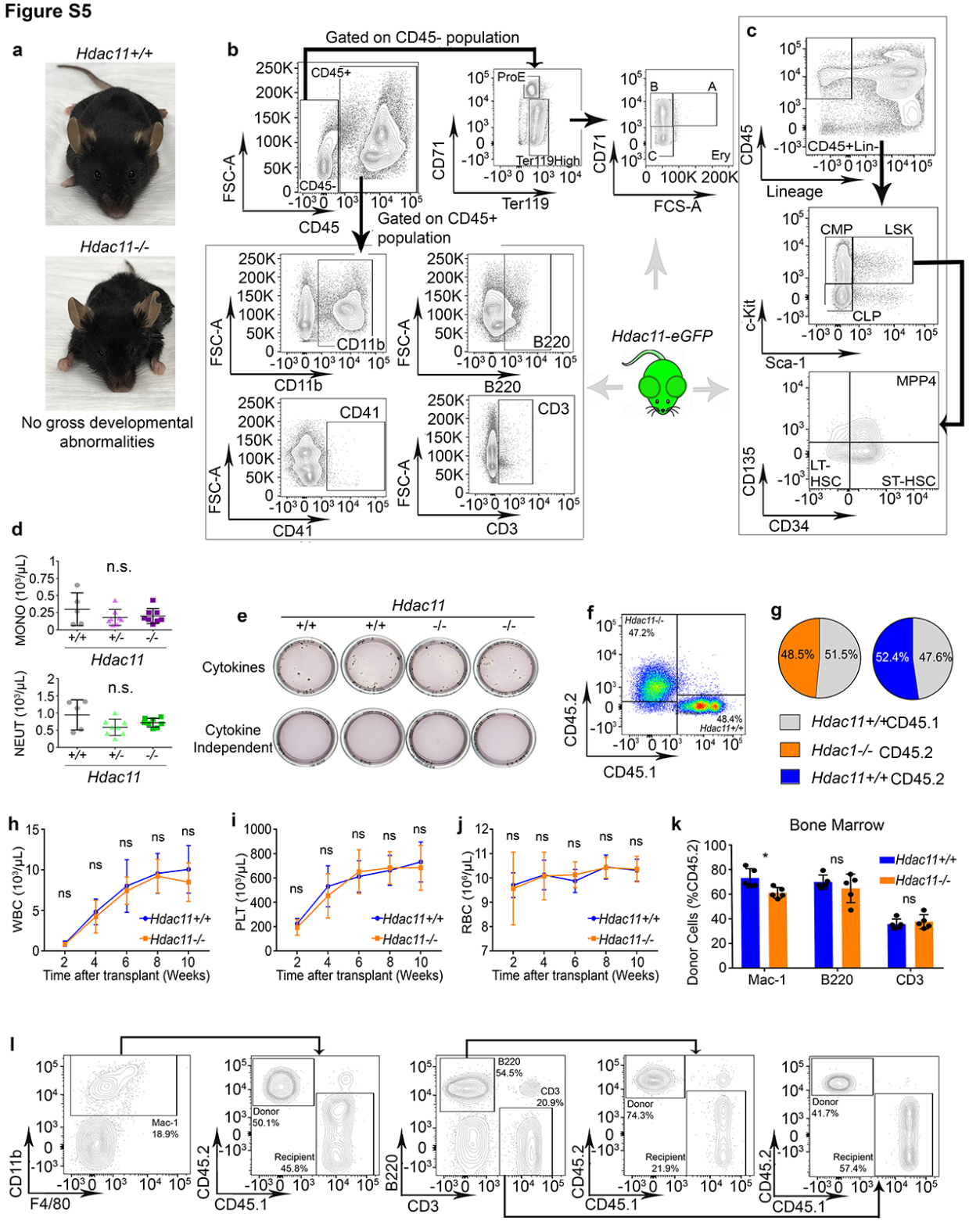


Extended data shown in Table S4

Supplementary Fig. S4 (Relevant to data shown in Fig. 4). Microarray analysis was conducted on HEL92.1.7 cells treated with 8 μM of the HDAC11 inhibitor FT234 or the inactive compound FT650 for 6, 12 and 24 hr, followed by total RNA extraction using the RNeasy Micro Kit (Qiagen). 100 ng of total RNA was converted into cDNA and amplified and biotin-labeled using the Ambion Message Amp Premier Kit according to manufacturer's protocol (ThermoFishser Scientific, Waltham, MA). Hybridization, staining, and scanning of the Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA) was performed as outlined in the Affymetrix technical manual as detailed previously[4]. Scanned output files were visually inspected for hybridization artifacts and then analyzed using Affymetrix Expression Console v 1.4.1 utilizing the MAS 5.0 algorithm. Principle Component Analysis and unsupervised hierarchical clustering were performed. Statistical analysis was performed using two-way ANOVA. Gene lists with 2-fold differential expression and false discovery rate (FDR) ≤0.05 between the control and HDAC11 inhibitor-treated cells were compared. Gene Ontology (GO) analysis was performed on genes differentially expressed in HDAC11 inhibitor-treated cells using MetaCore GeneGo data analysis tool (Clarivate Analytics). The microarray data have been deposited to the Gene Expression Omnibus database under accession number GSE81725. HDAC11 inhibitor-induced changes in transcription and cell cycle profile of MPN cells. (**a**) HEL92.1.7 cells were treated with an HDAC11 selective inhibitor FT234 or inactive control FT650 for 6h, 12h and 24h, following which RNA was extracted for microarray analysis. Quality and reliability of the microarray data were evaluated by principal component analysis (PCA). **(b)** Microarray data were analyzed using

Partek software, showing differentially expressed genes between FT234-treated cells and control compound treated cells. Differentially-expressed genes induced by FT234 treatment were subjected to gene set enrichment analysis (GSEA). Plots indicate the maximum enrichment score (ES), ranked list metric, and the leading-edge subset. Scores of positively and negatively correlated genes in each pathway are indicated. Raw output files provided in Supplementary Table S4. **(c)** Heatmap of differentially expressed cell cycle regulated genes. **(d-i)** Cell cycle analysis of HEL92.1.7 MPN cells treated. Cells treated with DMSO **(d)** or Panobinostat (10 nM) **(e)** with data summarized for 3 and 10 nM in **(f)**. Cell cycle analysis plots of DMSO-treated cells **(g)** compared to the CDK1 inhibitor RO3306 at 3.6 μM **(h)** and summarized for two doses 1.8 and 3.6 μM a as indicated for 12 hrs **(i)**. Data analyzed using ModFit LT software indicate percentage of cells in G1, S, and G2 phases of the cell cycle. Multiple t-test (compared to DMSO in each group) was performed for statistical analysis, non-significant = n.s., **p< 0.01, and **** p<0.0001

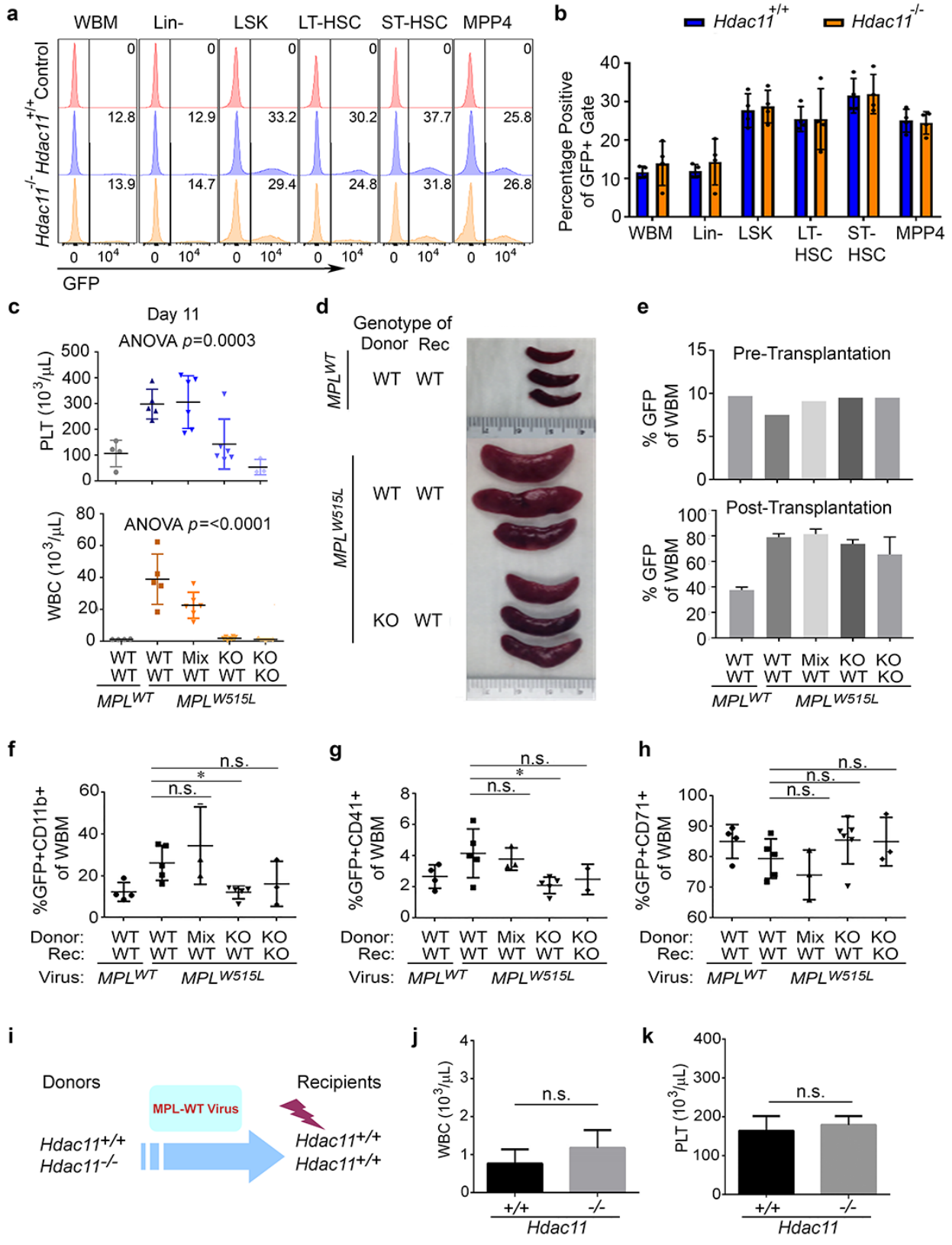
Figure S5


a
Hdac11+/+
Hdac11-/-
No gross developmental abnormalities
b
Gated on CD45- population
CD45+
CD45-
FSC-A
CD45
ProE
Ter119High
CD71
Ter119
B
A
C
Ery
FCS-A
Gated on CD45+ population
CD11b
B220
CD41
CD3
Hdac11-eGFP
c
CD45+Lin-
Lineage
CD45
CMP
LSK
CLP
c-Kit
Sca-1
MPP4
LT-HSC
ST-HSC
CD135
CD34
d
MONO (10³/μL)
NEUT (10³/μL)
n.s.
+/+
+/-
-/-
Hdac11
e
Hdac11
Cytokines
Cytokine Independent
f
Hdac11-/- 47.2%
48.4% Hdac11+/+
CD45.2
CD45.1
g
48.5%
51.5%
52.4%
47.6%
Hdac11+/+CD45.1
Hdac1-/- CD45.2
Hdac11+/+ CD45.2
h
WBC (10³/μL)
Time after transplant (Weeks)
Hdac11+/+
Hdac11-/-
i
PLT (10³/μL)
j
RBC (10⁶/μL)
k
Bone Marrow
Donor Cells (%CD45.2)
Mac-1
B220
CD3
l
CD11b
F4/80
Mac-1 18.9%
Donor 50.1%
Recipient 45.8%
B220 54.5%
CD3 20.9%
Donor 74.3%
Recipient 21.9%
Donor 41.7%
Recipient 57.4%

Supplementary Fig. S5 (Relevant to data shown in Figure 5). HDAC11 is expressed in mouse bone marrow cell subsets and *Hdac11* loss does not interfere with normal hematopoiesis. (**a**) *Hdac11*$^{-/-}$ mice exhibit no gross physical or developmental abnormalities compared to *Hdac11*$^{+/+}$ mice. **(b-c)** Bone marrow was harvested from *Hdac11-eGfp* mice (n=4) and *Hdac11* expression (GFP^{+} cells) in mature **(b)**, stem **(c)**, and progenitor **(c)** populations was analyzed via flow cytometry. **(d)** Monocyte (MONO) and neutrophil (NEUT) counts of *Hdac11*$^{+/+}$ (n=5) (n.s.), *Hdac11*$^{+/-}$ (n=9), and *Hdac11*$^{-/-}$ (n=9) mice (eight weeks old) are shown from these experimental groups. No significant differences were observed using an ANOVA followed by Tukey's multiple comparison with p-value <0.05. **(e)** *Hdac11*$^{+/+}$ (n=2) and *Hdac11*$^{-/-}$ (n=2) whole bone marrow cells were cultured in cytokine-supplemented and cytokine-free methylcellulose medium. Colony numbers after 7 days in culture. Data represents one of two independent experiments. **(f-l)** Bone marrow cells (1×10^{6}) from CD45.2 *Hdac11*$^{+/+}$ (n=4) or *Hdac11*$^{-/-}$ (n=4) mice were transplanted into lethally irradiated recipient mice (n=15 recipients/genotype) along with CD45.1 (1×10^{6}) recipient bone marrow cells. **(f-g)** Donor (CD45.2^{+}) and recipient (CD45.1^{+}) cells were mixed in a 1:1 ratio and the percentage of each in the injection was determined by flow cytometry. **(h-j)** White blood cells **(h)**, platelets **(i)**, and red blood cells **(j)** were evaluated for ten weeks after transplantation. **(k)** Donor cell reconstitution in the myeloid (Mac-1^{+}), B (B220^{+}), and T (CD3^{+}) cell lineages in the bone marrow was analyzed via flow cytometry. Statistical analysis was performed using multiple two-tailed unpaired t-test. **** p<0.0001, *** p<0.001, ** p<0.01, *p<0.05. **(l)** Gating Strategy to evaluate donor cell engraftment in

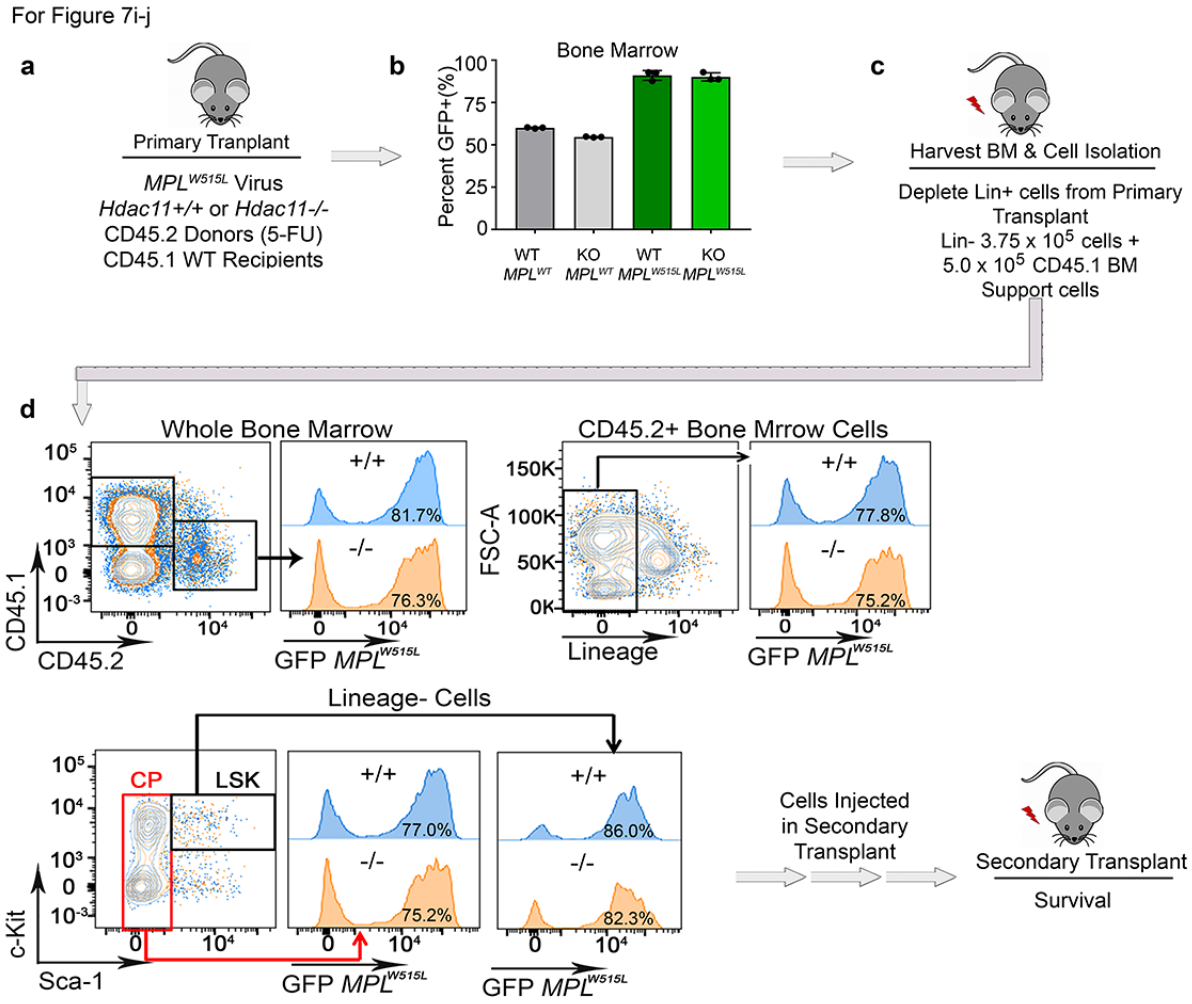
peripheral blood. After excluding debris, doublets, and dead cells, Mac-1, B220, or CD3 populations were gated on, and donor and recipient populations were distinguished by CD45.2 and CD45.1, respectively. Statistical analysis performed using multiple two-tailed unpaired t-test. ****p<0.0001, *** p<0.001, **p<0.01, *p<0.05.

Figure S6


a
WBM
Lin-
LSK
LT-HSC
ST-HSC
MPP4
Hdac11+/+ Control
Hdac11-/-
12.8
12.9
33.2
30.2
37.7
25.8
13.9
14.7
29.4
24.8
31.8
26.8
GFP
b
Hdac11+/+
Hdac11-/-
Percentage Positive of GFP+ Gate
WBM
Lin-
LSK
LT-HSC
ST-HSC
MPP4
c
Day 11
ANOVA p=0.0003
PLT (10^3/μL)
ANOVA p=<0.0001
WBC (10^3/μL)
WT WT
WT WT
Mix WT
KO WT
KO KO
MPLWT
MPLW515L
d
Genotype of Donor Rec
MPLWT
WT WT
MPLW515L
WT WT
KO WT
e
Pre-Transplantation
% GFP of WBM
Post-Transplantation
% GFP of WBM
f
%GFP+CD11b+ of WBM
n.s.
*
Donor:
Rec:
Virus:
g
%GFP+CD41+ of WBM
h
%GFP+CD71+ of WBM
i
Donors
Hdac11+/+
Hdac11-/-
MPL-WT Virus
Recipients
Hdac11+/+
Hdac11+/+
j
WBC (10^3/μL)
+/+
-/-
Hdac11
k
PLT (10^3/μL)

Supplementary Fig. S6 Chimeric transplantation with *Hdac11*$^{-/-}$ donors attenuates *MPL*^{W515L}-induced disease (Relevant to data shown in Figure 7). (**a-b)** Wild-type (WT, *Hdac*$^{+/+}$) and *Hdac11 -/-* bone marrow was infected with *Gfg-MPL*^{W515L} recombinant virus. (**a**) Histograms of % GFP positive cells by genotype in WBM (whole bone marrow), Lin- cells, LSK, LT-HSC, ST-HSC and MPP4 from one mouse (percent positive indicated compared to non-transformed cells used for control). (**b**) Summary of data of four biological replicates from one independent experiment repeated three times (4x total) with similar results observed. (**c-h**) Recipients were transplanted with *MPL*^{WT}- or *MPL*^{W515L}-expressing BM, or with a 1:1 mixture *MPL*^{W515L}-expressing *Hdac11*$^{+/+}$ and *Hdac11*$^{-/-}$ BM donors. *Hdac11*$^{-/-}$ recipients also received *MPL*^{W515L}-transduced *Hdac11*$^{-/-}$ bone marrow to assess potential effects of *Hadc11* deficiency on the bone marrow niche. (**c)** Disease-progression was measured on day 11 via PLT and WBC counts. (**d**) Spleen size and (**e**) Transduction efficient was determined by GFP expression via flow cytometry prior to injection (pre-transplantation) and at the endpoint on day 20 (post-transplantation). (**f-h**) Twenty days after transplant all 5 transplanted groups were examined for the percentage of mature populations in whole bone marrow (WBM) including GFP+CD11b+ (**f**), GFP+CD41+ (**g**), and GFP+CD71+ (**h**) to access HDAC11's role in MPN (after gating on live cells). Statistical analysis is shown *p=0.05. Data represents the percentage of cells on day 20. (**i**) Schema for matched transplantation of *MPL*^{WT} virus into *Hdac11*$^{+/+}$ and *Hdac11*$^{-/-}$ for reconstitution of normal hematopoietic cells white blood cell counts (WBC) (**j**) and platelet counts (Plts) (**k**) on day 38.

Figure S7



Supplementary Fig. S7. Relates to data shown in Fig. 7h. *Hdac11* deficiency maintains its ability to improve survival in secondary transplantation of MPL^{W515L}. (**a**) $Hdac11^{+/+}MPL^{WT}$, $Hdac11^{-/-}MPL^{WT}$, $Hdac11^{+/+}MPL^{W515L}$ and $Hdac11^{-/-}MPL^{W515L}$ bone marrow cells were transplanted into lethally irradiated recipients (n=10 per group). (**b**) Disease progression was determined by GFP expression in the bone marrow. Statistical analysis performed using two-tailed unpaired t test. **** P <0.0001, *** P<0.001, ** P <0.01, *P<0.05. (**c**) After diseased had progressed to similar levels, lineage negative bone marrow cells from $Hdac11^{+/+}MPL^{W515L}$

and *Hdac11*$^{-/-}$*MPL*^{W515L} were enriched and transplanted (3.75x10^5, i.v.) into secondary recipient with support recipient whole bone marrow (5x10^5, i.v.). (**d**) GFP expression in CD45.2 Lin-, LSK and committed progenitor populations was examined prior to secondary transplant.

References:

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4. Mailloux AW, Zhang L, Moscinski L, et al. Fibrosis and subsequent cytopenias are associated with basic fibroblast growth factor-deficient pluripotent mesenchymal stromal cells in large granular lymphocyte leukemia. *J Immunol*. 2013;191(7):3578-3593.