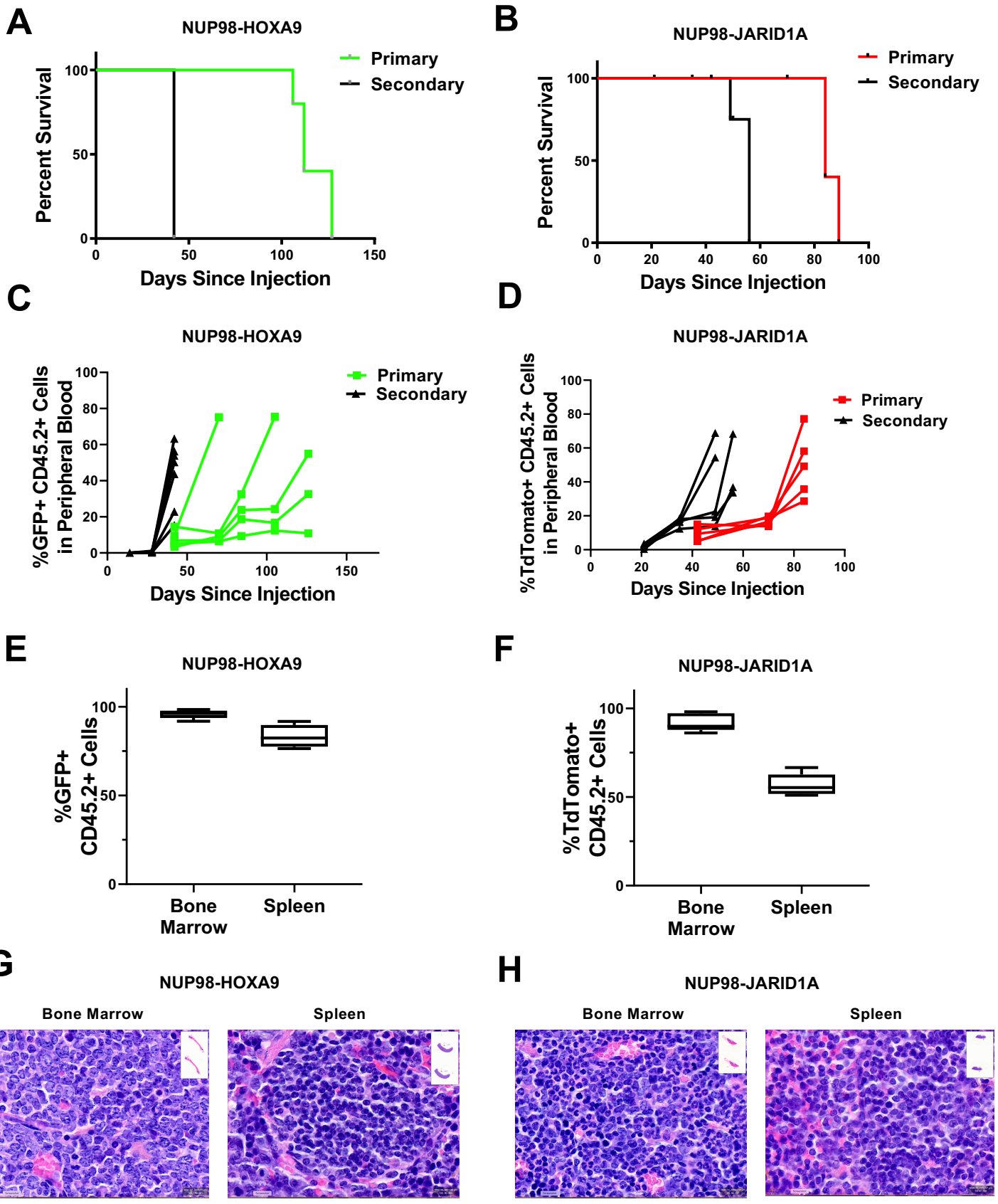
Supplemental Figure 1



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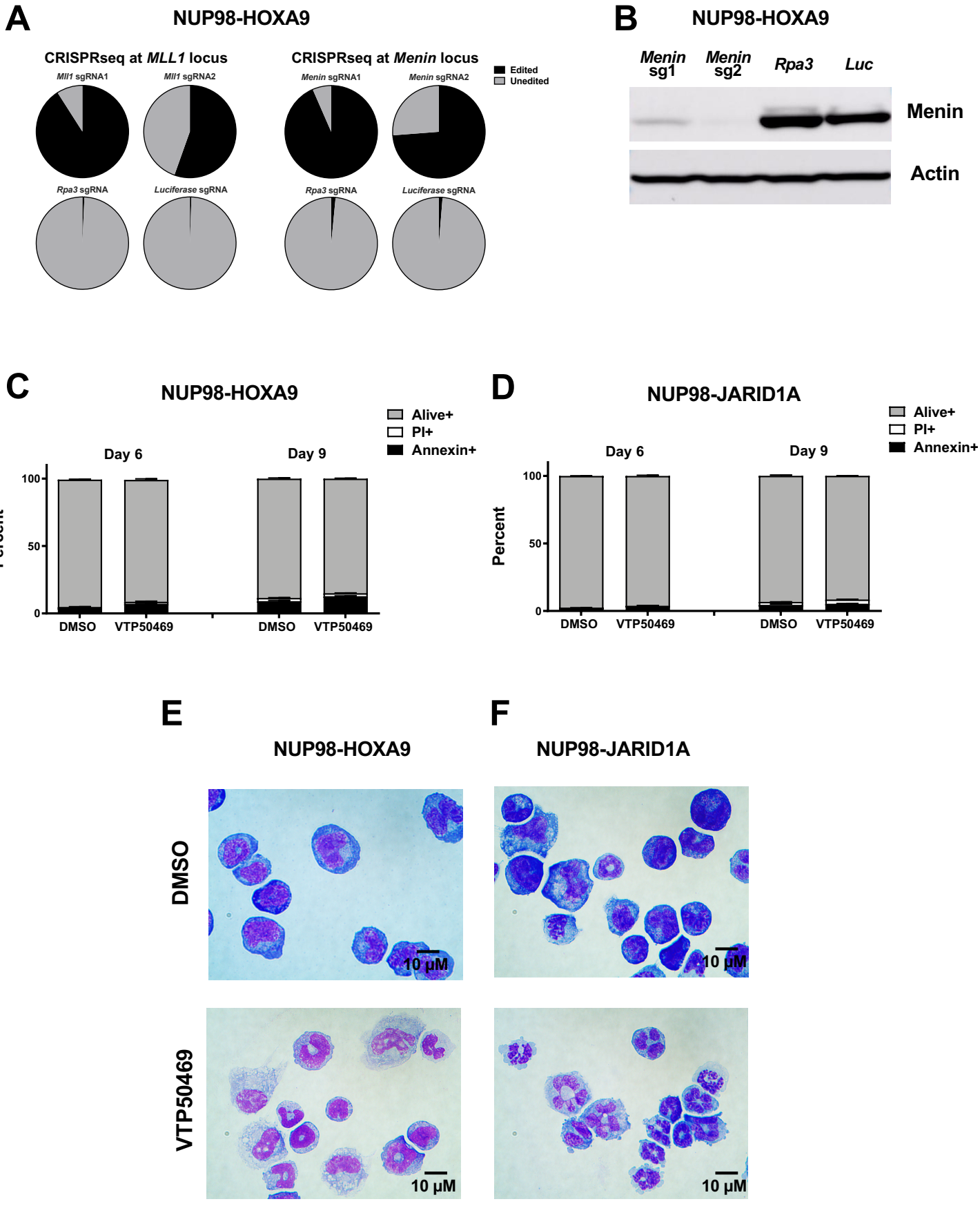

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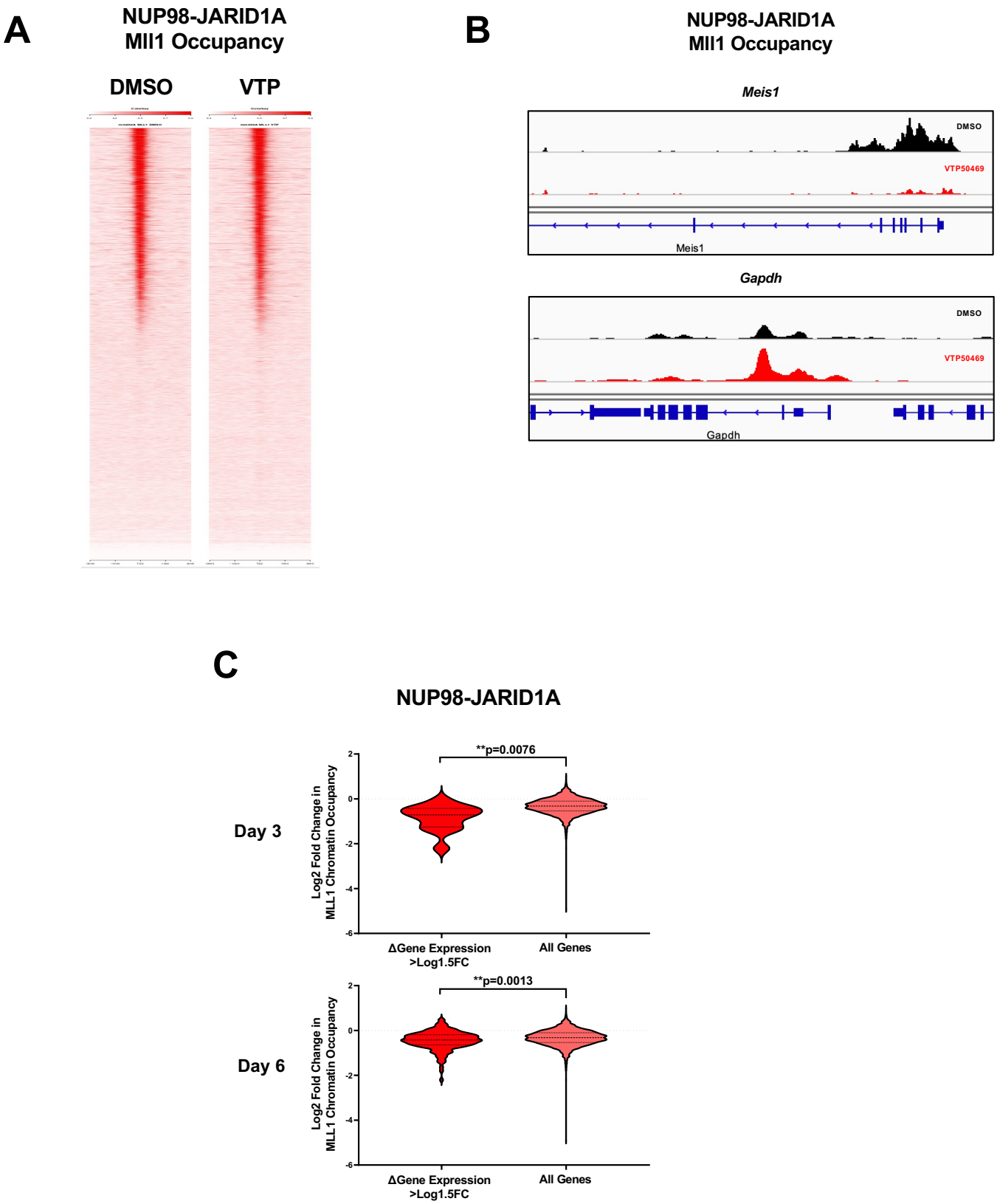

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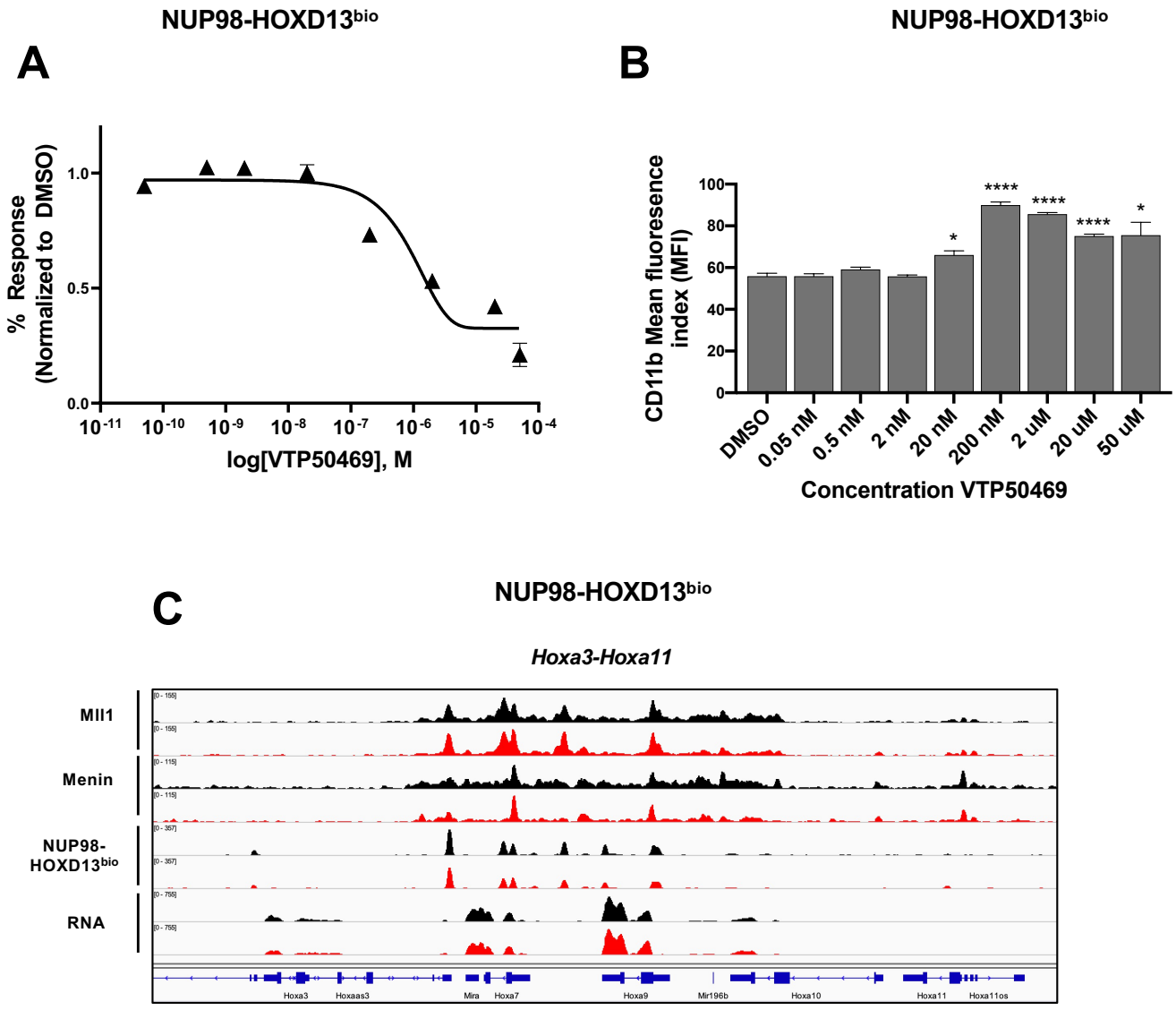
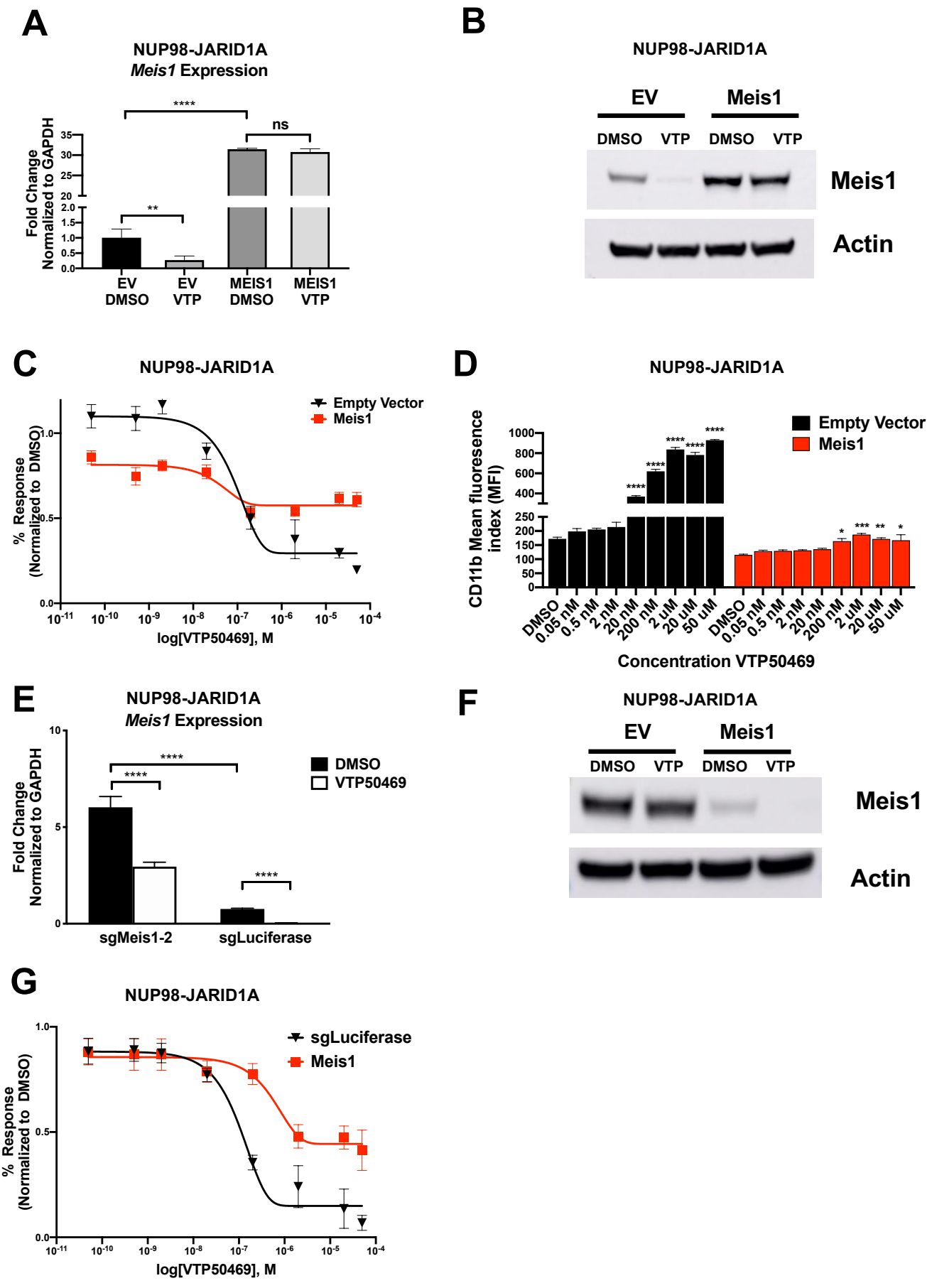
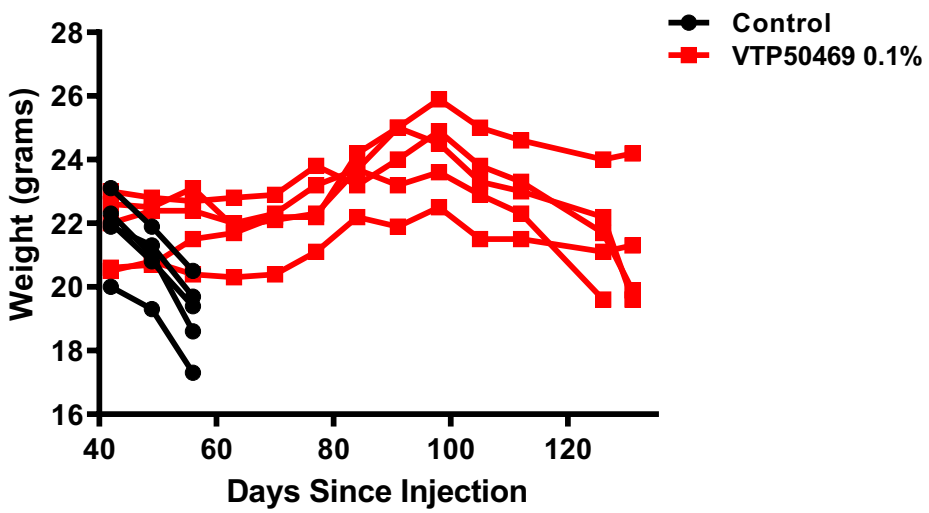


Supplemental Figure 5



Supplemental Figure 6

A



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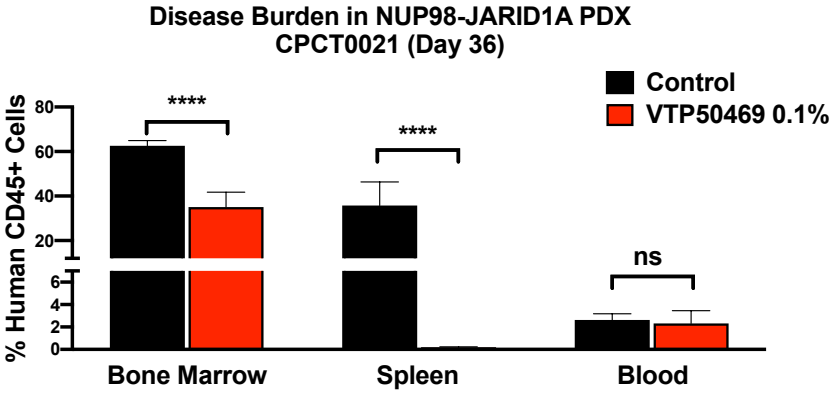
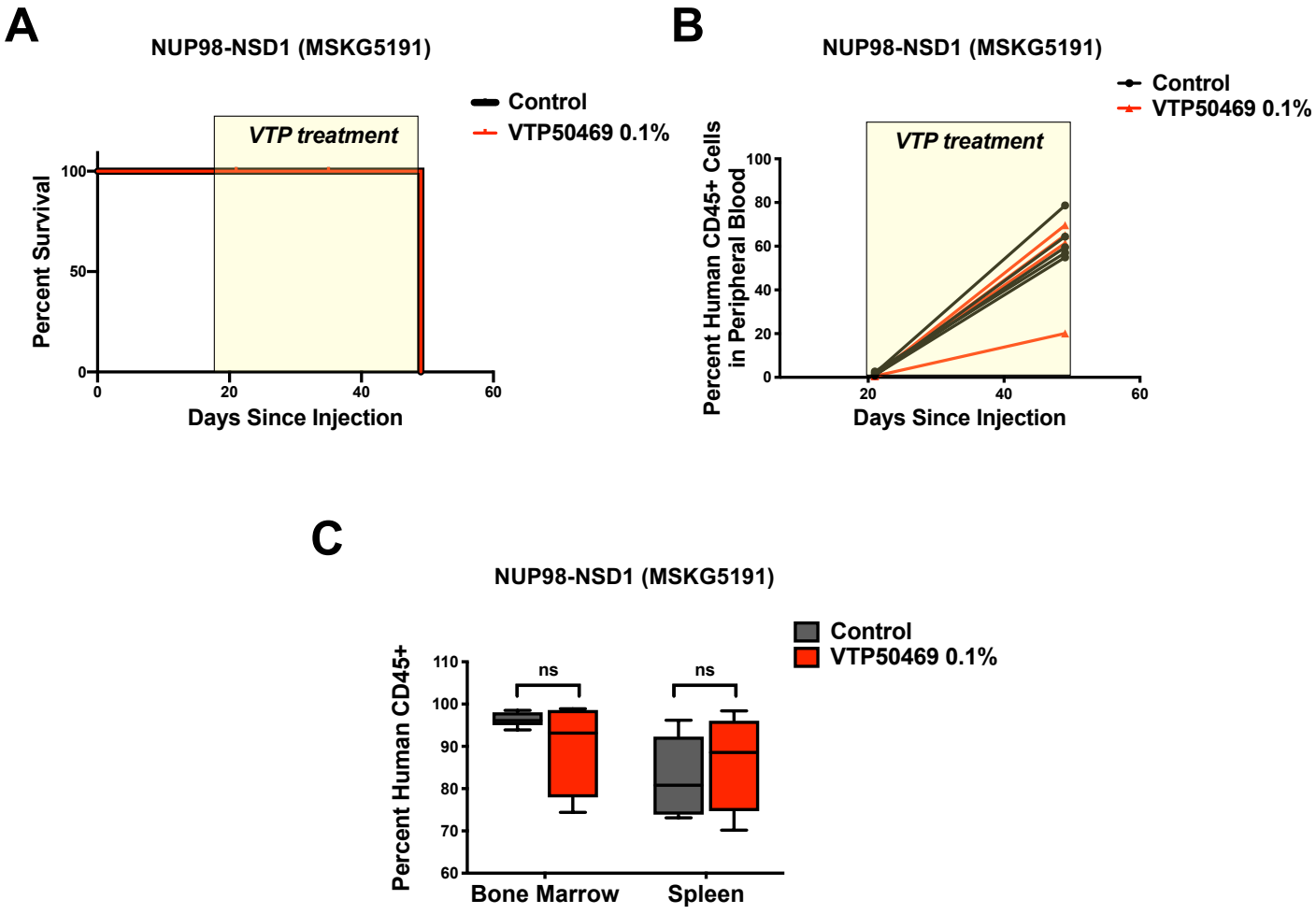
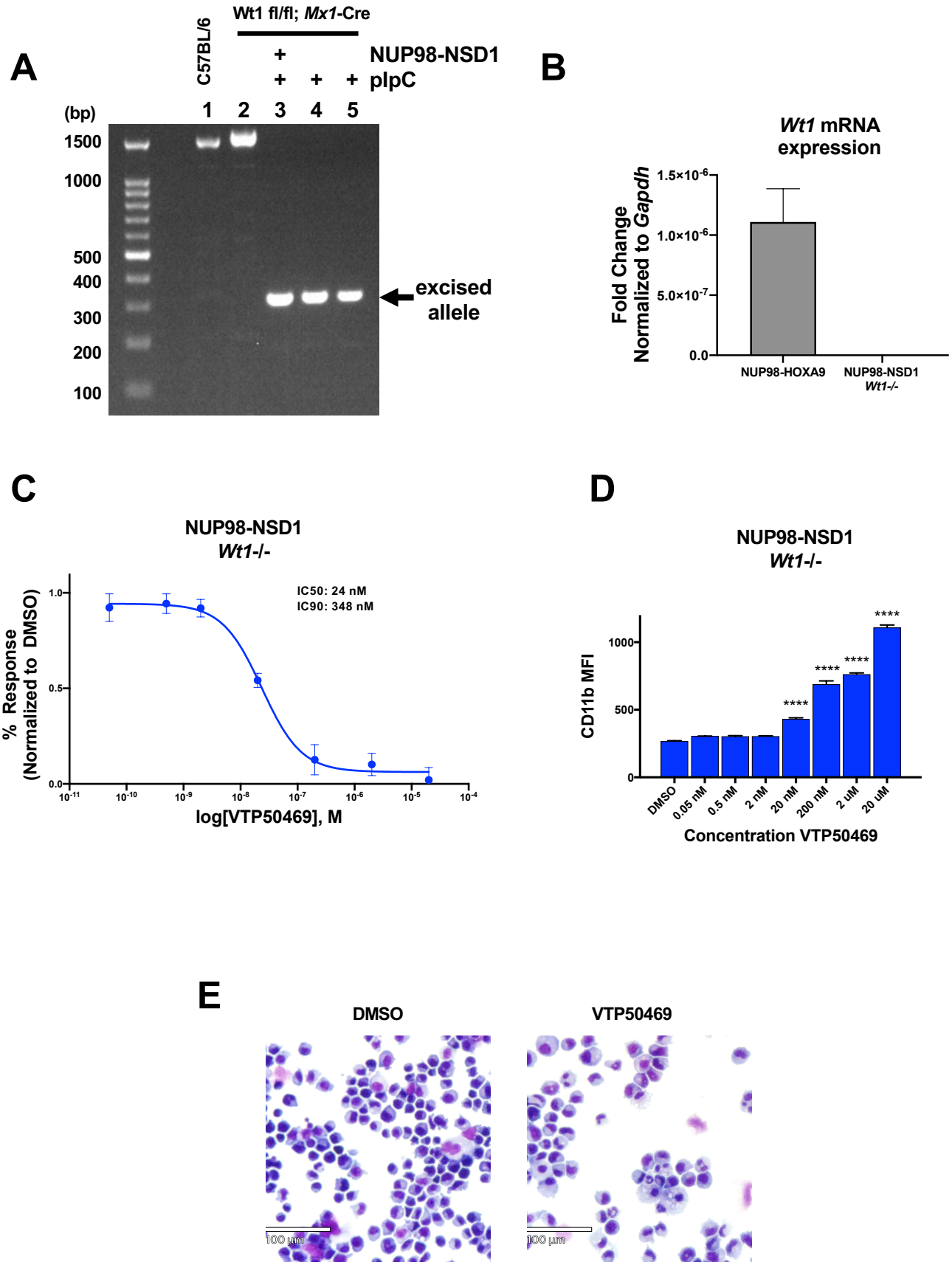


Supplemental Figure 7

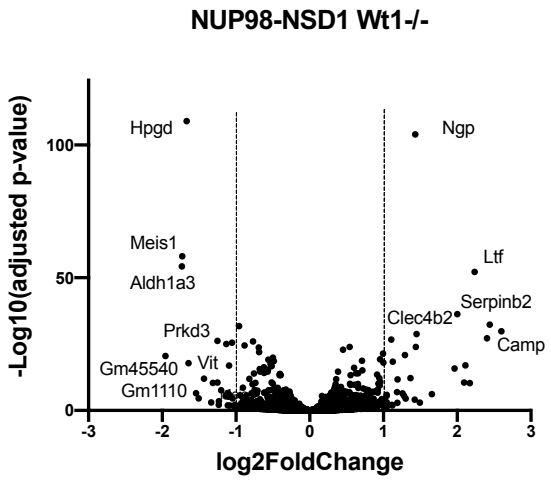


Supplemental Figure 8



Supplemental Figure 8, continued

F



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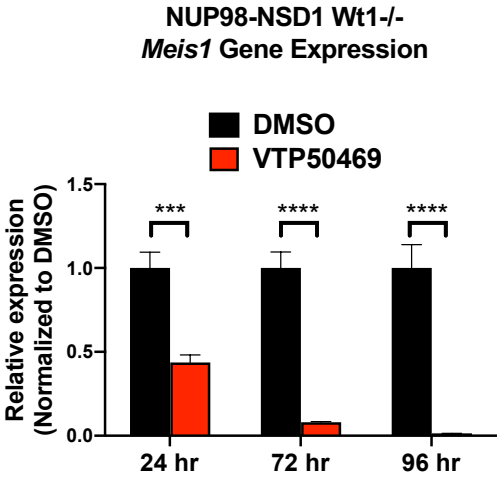

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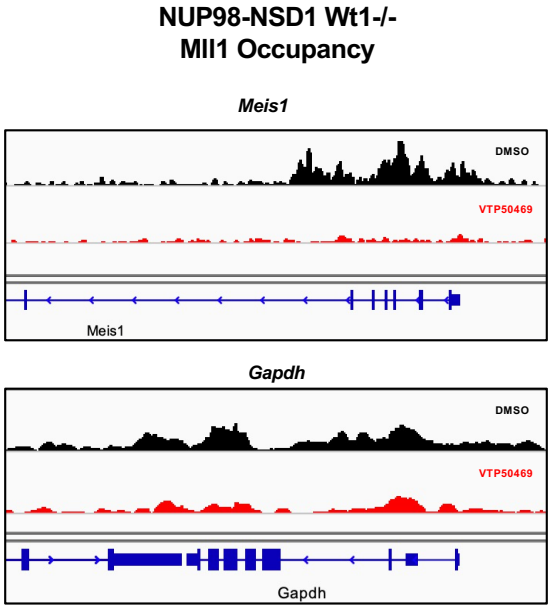
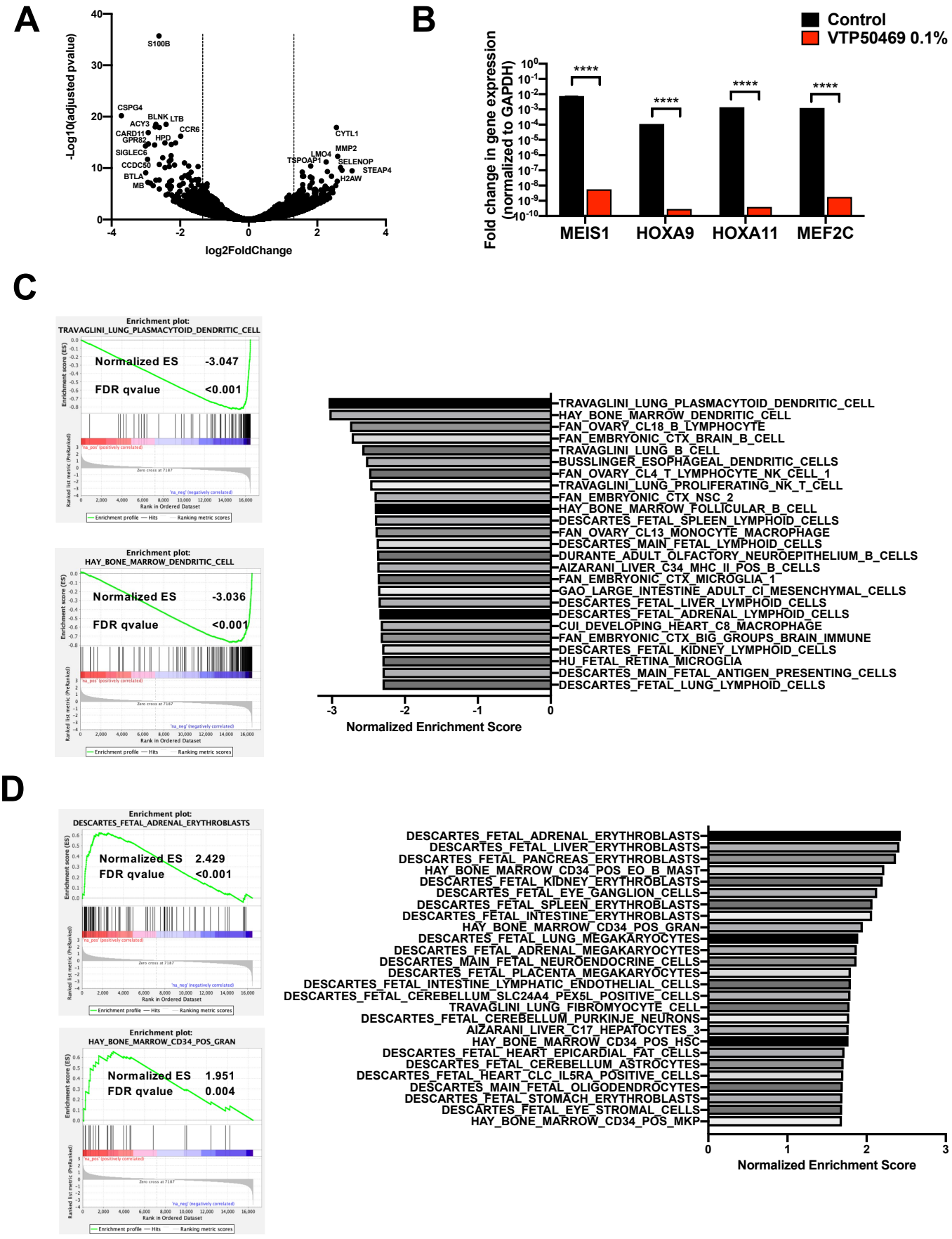
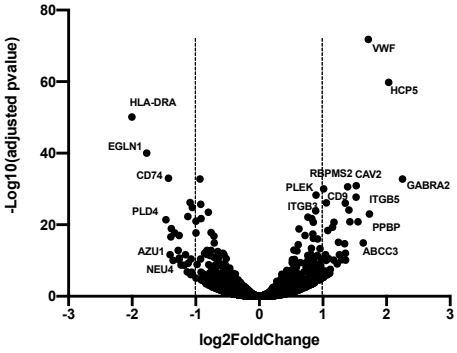


Supplemental Figure 9



Supplemental Figure 10

A



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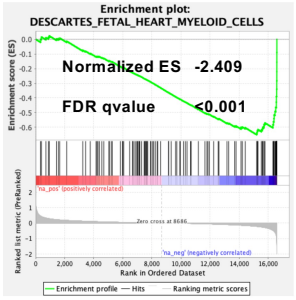

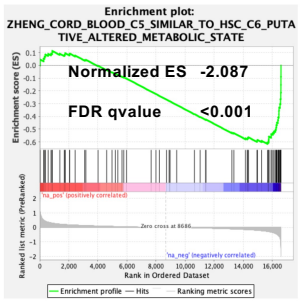

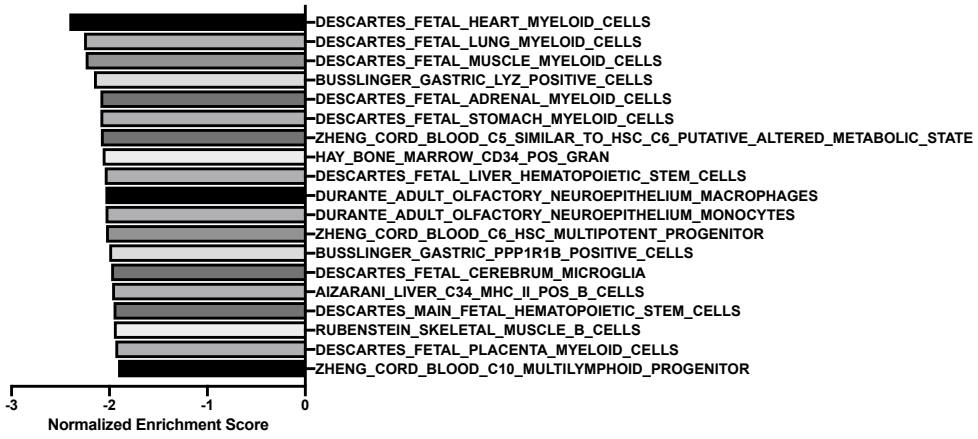

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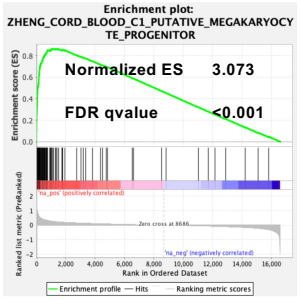
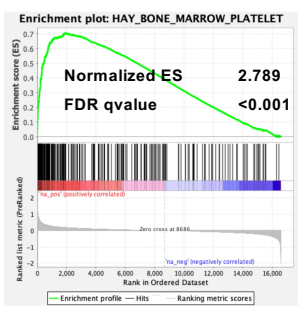
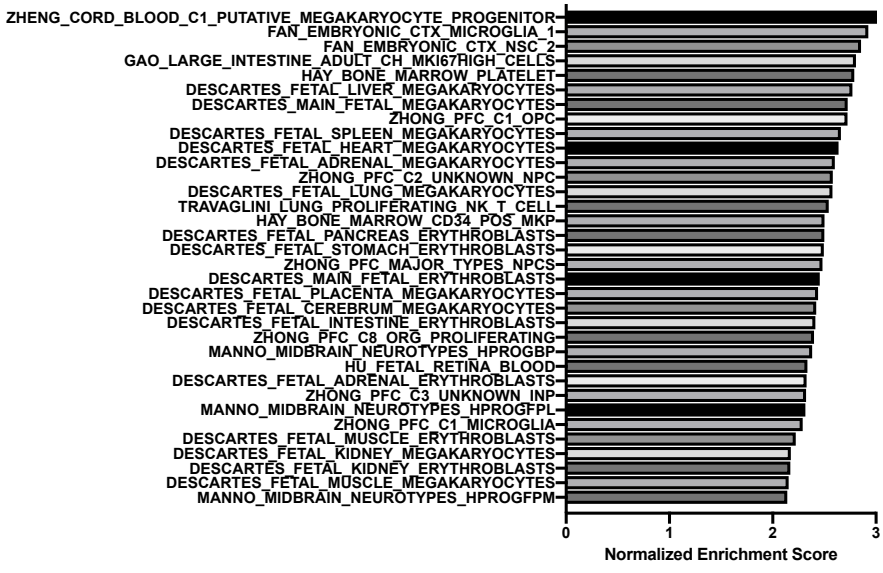
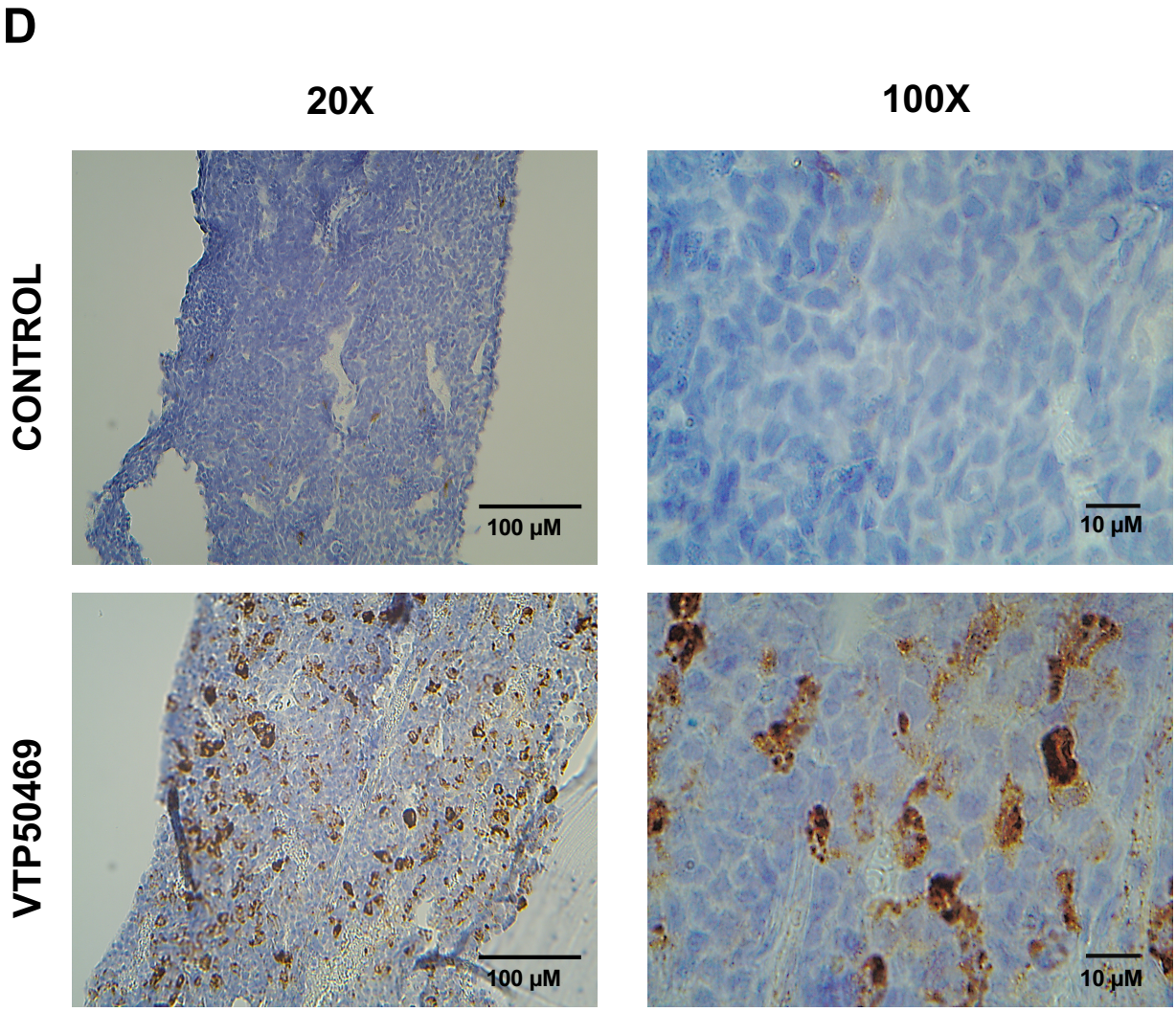






Supplemental Figure 10, cont'd



Supplemental Table 1: Small guide RNA sequences for *in vitro* CRISPR competition assays.

Gene	CRISPR targeting sgRNA
MLL1 sg1	5’ GGCAGCCGTTAGACCTCGAA 3’
MLL1 sg2	5’ GATCCGGGGTCCTCGAACAG 3’
Menin sg1	5’ TCTGCGCTCTATCGACGACG 3’
Menin sg2	5’ ACGTCGTCGATAGAGCGCAG 3’
RPA3	5’ TGTTACCACAGTATATCGAC 3’
Luciferase	5’ AGATTCTAAAACGGATTACCA 3’

Supplemental Table 2: Genes with >Log1.5 fold-change in expression in NUP98-JARID1A mouse leukemia cells treated with VTP50469

NUP98-JARID1A ΔGene Expression >Log1.5FC				
Day 3	**Day 6**			
Man1a	Fasn	Fut8	Dna2	Meis1
Fut8	Prrc2c	Clic4	Vps13a	Qser1
Bambi	Zbtb25	Srl	Setx	Gart
Frat2	Slc16a1	Ric1	Atp10a	Rdx
Pik3ip1	Erbin	2610021A01Rik	Frmd6	Six1
Epha7	Mtf2	Ppip5k2	Gnptab	L2hgdh
Ccno	Slc7a1	Jmjd1c	Fam169b	Cnnm2
Nlrc5	Kansl1l	Osbpl3	Slc4a7	Plpp5
Itpr1	Rsbn1	Syne3	Alms1	Tnfaip6
Nrg4	N4bp2	Fam185a	Klhdc2	Iars
Chd9	Psme4	Cdkn2c	Epha7	Slc7a5
Fmnl2	Brca2	Opn3	Uhrf1bp1	Jmy
Meis1	Chst11	Aldh7a1	Ankrd28	Arid5a
	Ptpn4	Trim37	Nsmaf	Cnot1
	Tmx4	Frat2	Igtp	Usp31
	Dyrk3	Wee1	Wrn	Nsd1
	Vps13c	Adam17	Pcgf5	Arhgap21
	Fancm	Tasp1	Kif17	
	Rev3l	Pus7	B4galt6	
	Nup210	Sacs	Ttll4	
	Zfp266	Nedd4	Clip1	
	Me2	Thap2	Sesn1	
	Ston2	Trim59	Runx2	
	Gbp7	Saraf	Gfi1	
	Katnal1	Pik3ip1	Acaca	
	Nr2c2	Btg3	Ptar1	
	Cep350	Usp34	Itpr1	
	Ranbp2	Zfp800	Myb	
	Il15ra	Shmt1	Prkd3	
	Xrcc2	4932438A13Rik	Chsy1	
	Bcl2	Zfp395	Slc7a6	
	Rpusd2	Thada	Atp8b4	
	Tex2	Cep192	Maml3	
	Kdm2b	Rai1	Cebpa	
	Msh2	Msi2	Chd9	
	Tfrc	Peli1	Ak4	
	Uck2	Itga4	Fmnl2	
	Abhd17b	Tiam1	Kctd1	
	Ern1	Fndc3b	Nav2	

Supplemental Table 3: Genes with >Log1.5 fold-change in expression in NUP98-HOXA9 mouse leukemia cells treated with VTP50469

NUP98-HOXA9 ΔGene Expression >Log1.5FC		
Day 3	**Day 6**	
Tulp3	Depp1	Bmp2k
Lpxn	Tgm2	Man1a
Pdlim5	Hmces	Phtf2
Bcl2	Adam17	Zfp608
Calcrl	Ston2	Rnf19b
Tox	Tulp3	Cdk17
Phtf2	Gbp2	Snx24
Msi2	Nt5c3	Ern1
Meis1	Six1	Msi2
Pbx3	Nxpe3	Meis1
Zcchc7	Mov10	Pcdh7
	Ptger4	Pbx3
	Papss2	Kansl1l
	Pde4b	Zcchc7
	Lpxn	Bbx
	H2-T23	Rsad2
	Samsn1	Socs5
	Epsti1	Ppp1r15a
	Psmb8	Prepl
	Car2	Trib1
	Atp10a	Gm35315
	Cuedc1	H2-Oa
	Dusp5	Dyrk3
	Dtnb	Tef
	Clip1	Tpm1
	Mns1	Lyrm4
	Reps1	Trp53bp2
	Hoxb7	Hdc
	Endod1	Tnfaip6
	Car3	Fyn
	Sesn1	Tnfrsf9
	Nedd4	
	Hoxb6	
	Pdlim5	
	Bcl2	
	Maml3	
	Calcrl	
	Iqgap2	
	Adk	
	Tgif1	
	Tox	

Supplemental Table 4: Genes with >Log2 fold-change in expression in NUP98-HOXD13^{bio} mouse leukemia cells treated with VTP50469

NUP98-HOXD13^{bio} Targets ΔGene Expression >Log2FC	All NUP98-HOXD13^{bio} Targets						
Mef2c	Tsc22d2	Fam49b	Rasal2	Smyd3	Dusp6	Spock3	Bcas3
Zcchc7	Stag1	Foxp1	Bcl11a	Tcf4	Kctd12	Lrrc69	Hhat
Meis1	Pcdh7	Klhl28	Anapc10	Auts2	Olfr592	Fbxl13	Tor1aip2
Ssbp2	Cog5	Hecw2	Samsn1	Zfp52	Nmbr	Zfp800	Crisp1
Pbx3	Mef2c	Ankrd28	Kifap3	Numb	Upp1	Wdpcp	Mertk
Eya1	9930021j03rik	Tcf12	Tcf20	Zfp64	Fam117b	Pten	Mreg
Foxp1	Magi3	Lrif1	Zfp608	Zfp935	Nol4	Fnbp1l	Ankrd36
Rab38	Zcchc7	Nr4a2	Kif11	Eea1	Apbb1ip	Tmem38b	Ankrd36
Cp	Vps13a	Pgm2l1	Ccser1	Trdmt1	Casd1	Snx6	Prlr
Fars2	Kansl1l	Rab38	Calcrl	Macc1	Ahr	Dthd1	Mctp1
Glra3	Fut8	Pik3r1	Csnk1g1	Tbl1xr1	Rnf180	Tmprss11f	Tbc1d32
Calcrl	Galnt1	Qk	Angpt1	Osgin2	Zfp820	Tex14	Naip1
Mecom	Akap13	Mbnl1	Hoxa9	Il22ra2	Hgf	Gsk3b	Dab2
Tcf4	Ascc3	Stim2	9130008f23rik	Arhgap18	Rpia	Otud4	Itgb8
Alyref2	St8sia4	Usp47	Homer1	Zfr	Cdc42bpa	Max	Otop1
B230118h07rik	Gtdc1	Cacul1	Ddx4	Auh	Kcnj2	Spdya	Sos1
Olfr1259	Dennd1b	Pds5a	Wdr44	Muc13	Flrt2	Ptgs2	Wfdc17
Pcdh17	Kcnrg	Map4k5	Scfd2	Rps6ka5	Casp4	Hmbox1	Ptpn3
Efcab5	Fgd4	Stam	Tmem135	Kcnj16	Ptprd	Arhgap26	Klf6
	Strbp	Rsbn1l	Nufip1	Pip4k2a	Snx13	Adrb2	Cbwd1
	Meis1	Nptn	Specc1	Tespa1	Olfr161	Pecr	Tnfsf18
	Nipbl	Hhip	Prpf39	Asph	Dock8	Map3k7cl	Chd7
	Skap2	Cdk6	Ripk2	Sos2	Zfp868	Uba3	Dusp22
	Ppp3cb	Fars2	Neil3	Hormad2	Alyref2	Car8	S1pr3
	Ssbp2	Mpp6	Papss1	Hdac9	Foxk1	Dmxl2	Oprm1
	Rgs18	Fkbp5	Tbc1d5	Mapkap1	Yipf7	Ercc3	
	Runx2	Tfec	Micu3	Myb	Akr1e1	B230118h07rik	
	Pbx3	Exoc6	4930544g11rik	Rassf6	Phf3	Rptor	
	Mtx2	Plxdc2	Mecom	Fbxo33	4930467e23rik	Rb1	
	Dennd1a	Ppp3ca	Tec	Olfm3	Crisp3	Cdh9	
	Baz2b	Klhl24	Fcho2	Foxp2	Triqk	Clec5a	
	Eya1	Rap1a	Tle4	Krr1	Gmds	Vmp1	
	Zc3h12c	Cenpk	Exoc2	Hibadh	Cdh12	Nedd9	
	Strn	Nf1	Csnk1g3	Tax1bp1	Dram1	Olfr1259	
	Cux1	Cul3	Map2k4	Ets1	Rbm46	Phf8	
	Dach1	Prkacb	Gm597	Eapp	Immp2l	Cltc	
	Vps54	Pla2g4a	Gcnt4	Chl1	Fbxw17	Fggy	
	Btbd10	Ncoa1	Hoxa7	Pcmtd1	Hoxb8	Pdgfc	
	Dzip3	Ahi1	Rbm33	Hoxc5	Vps45	Slfn14	
	Gbe1	Adk	Sgcz	Egln1	Gtf3c6	Pcdh17	
	Phip	Glra3	Hoxc4	Ick	Ptpn12	Efcab5	
	Mars2	Igip	Stpg2	Cdk19	Plekha5	Jazf1	
	Zc3h6	Scfd1	Baz1a	Pan3	Ncam2	Edil3	
	Gphn	Eml4	Pik3ap1	Grik2	Kat6b	Mbl2	
	Ezh2	Gab2	Rab3c	Bbx	Stim1	Ppp1r12a	

Supplemental Table 5: Small guide RNA sequences for *in vitro* CRISPRa *Meis1* rescue experiments.

Gene	CRISPRa targeting sgRNA
sgMeis1-1	5’ GCTGCAGCAAGTAGGCTCCT 3’
sgMeis1-2	5’ GCAAGTAGGCTCCTCGGCAG 3’
Luciferase	5’ AGATTCTAAAACGGATTACCA 3’

Supplemental Table 6: Genetic and phenotypic characteristics of patient-derived xenograft models

PDX Unique Identifier	Fusion Protein	Co-occurring mutations	Immunophenotype
CPCT0021	NUP98-JARID1A	none	Positive markers: CD45 dim, CD117 dim, CD4, CD35, CD38, partial HLA-DR, CD71, CD61 (weak) and CD41a (weak) Negative markers: CD34, nuclear TdT, B-lineage, T-lineage (other than CD4), cytoplasmic MPO, CD33.
NTPL-511	NUP98-NSD1	Retained introns in ABL1 and MLLT10 genes were identified along with missense variants in TCF3 and CCND3	Positive markers: CD45, CD117, CD13, CD33, CD34+ (partial), HLA DR+, CD11b (partial), CD7 (70%), CD38, CD71 Negative markers: CD11c, CD14, CD19, CD10, CD20, CD2, CD4, CD5, CD25 (6%, weak), CD41, CD56, CD62, CD64-/+ (5%, weak), glycophorin A-, cytoplasmic CD3-, cytoplasmic CD79a-, myeloperoxidase +/- (21%), TdT-
MSKG5191	NUP98-NSD1	BCORL1 (p.Y1692*, c.5076C>G) WT1 (p.S381* (c.1142C>A), ASXL1 (p.G1397S, c.4189G>A) IDH1 (p.R132G, c.394C>G) IDH1 (p.R132H, c.395G>A) FLT3 (p.E604_F605insGGEYDLKWE, c.1811_1812insAGGGGGTGAATATGATCTCAAATGGGA)	Positive markers: CD13, CD33, CD34, CD38, CD123, HLA-DR, CD4, CD45, CD71 and CD117. Negative markers: CD2, CD5, CD7, CD11b, CD14, CD15, CD16, CD19, CD25, CD56 and CD64.

Supplemental Figure Legends

Supplemental Figure 1: (A-B) Survival analysis of B6.SJL CD45.1 mice transplanted with mouse leukemia cells driven by NUP98-HOXA9 (**A**) or NUP98-JARID1A (**B**) and marked by CD45.2. (**C**) Percent GFP+ CD45.2+ leukemia cells as measured by flow cytometry in the peripheral blood of diseased mice bearing NUP98-HOXA9 leukemia cells. (**D**) Percent TdTomato+ CD45.2+ leukemia cells as measured by flow cytometry in the peripheral blood of diseased mice bearing NUP98-JARID1A leukemia cells. (**E-F**) Disease burden at the time of death for NUP98-HOXA9 (**E**) and NUP98-JARID1A (**F**) leukemia bearing mice, as measured by percent GFP+ CD45.2+ or TdTomato+ CD45.2 positive cells in the bone marrow and spleen. (**G-H**) Hematoxylin and eosin staining of bone marrow and spleen from NUP98-HOXA9 (**G**) and NUP98-JARID1A (**H**) leukemia bearing mice. n=5 mice per group.

Supplemental Figure 2: (**A**) Pie chart of percentage of CRISPR-edited sequencing amplicons from genomic DNA isolated from NUP98-HOXA9 mouse leukemia cells constitutively expressing Cas9 and small guide RNA (sgRNA) targeting *Mll1*, *Menin*, *Rpa3*, or *Luciferase*. (**B**) Immunoblot for Menin in NUP98-HOXA9 mouse leukemia cells constitutively expressing Cas9 and small guide RNA (sgRNA) targeting *Menin*, *Rpa3*, or *Luciferase*. (**C-D**) Cell death analysis in response to Menin-MLL1 inhibition as measured by flow cytometry of propidium iodide and annexin positive cells at days 6 and 9 of treatment with VTP50469 in NUP98-HOXA9 (**C**) or NUP98-JARID1A (**D**) mouse leukemia cells. (**E-F**) Cytological preparations of NUP98-HOXA9 (**E**) or NUP98-

JARID1A (**F**) mouse leukemia cells treated in vitro with 2 μM VTP50469 for 9 days. Images were acquired using an Olympus BX41 microscope with the 100X objective with acquisition software SPOT Version 5.1. Data are representative of 3 independent experiments.

Supplemental Figure 3: (**A**) Tornado plots depicting genome-wide MLL1 chromatin occupancy as determined by chromatin immunoprecipitation sequencing (ChIPseq) in mouse leukemia cells NUP98-JARID1A treated with DMSO or 2 μM VTP50469. (**B**) ChIPseq gene density plots showing changes in chromatin occupancy of MLL1 in reads per million (rpm) upon treatment with VTP50469 in mouse leukemia cells expressing NUP98-JARID1A (**C**) Violin plots depicting change in MLL occupancy for genes that display changes in gene expression versus all genes in NUP98-JARID1A after 3 days (top panel) or 6 days (bottom panel) of treatment with VTP50469. Data are representative of 2 independent experiments. One-way ANOVA was performed.

Supplemental Figure 4: (**A**) Mouse leukemia cells expressing NUP98-HOXD13^{bio} treated with an escalating dose curve of VTP50469. (**B**) Expression of CD11b on NUP98-HOXD13^{bio} mouse leukemia cells treated with an escalating dose curve of VTP50469. Data are representative of 3 independent experiments. One-way ANOVA with Dunnett's multiple comparisons test was performed, *P<0.05, ****P<0.0001 (**C**) ChIPseq and RNAseq gene density plots showing changes in chromatin occupancy of Mll1, Menin, NUP98-HOXD13^{bio} and mRNA expression at the *Hoxa* cluster in reads per

million (rpm) upon treatment with VTP50469 in mouse leukemia cells expressing NUP98-HOXD13^{bio}. Data are representative of 2 independent experiments.

Supplemental Figure 5: (**A**) Quantitative PCR for *Meis1* mRNA in NUP98-JARID1A mouse leukemia cells engineered to overexpress Meis1 and treated with DMSO or 2 μM VTP50469. Data are representative of 3 independent experiments. Two-way ANOVA with Dunnett's multiple comparisons test was performed, ***P*<0.01, *****P*<0.0001. (**B**) Immunoblot for Meis1 protein in NUP98-JARID1A mouse leukemia cells engineered and treated as in (**A**). (**C**) NUP98-JARID1A mouse leukemia cells engineered as in (**A**) and treated with an escalating dose curve of VTP50469. Data are representative of 3 independent experiments. (**D**) Expression of CD11b on NUP98-JARID1A mouse leukemia cells overexpressing Meis1 and treated with an escalating dose curve of VTP50469. Data are representative of 3 independent experiments. Two-way ANOVA with Dunnett's multiple comparisons test was performed, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. (**E**) Quantitative PCR for *Meis1* mRNA in NUP98-JARID1A mouse leukemia cells engineered to constitutively express dead Cas9 (dCas9) and transcriptional activators MS2, p65, and HSF1 with small guide RNAs targeting *Meis1* or luciferase and treated with 2 μM VTP50469. Data are representative of 3 independent experiments. Two-way ANOVA with Dunnett's multiple comparisons test was performed, *****P*<0.0001. (**F**) Immunoblot for Meis1 protein levels in cells engineered and treated as in (**E)**. (**G**) NUP98-JARID1A mouse leukemia cells engineered as in (**E**) and treated with an escalating dose curve of VTP50469. Data are representative of 3 independent experiments.

Supplemental Figure 6: (**A**) Weight in grams of NOG mice engrafted with a NUP98-JARID1A PDX (CPCT0021) for the duration of the experiment. n=5 mice per group (**B**) Disease burden in bone marrow, spleen, and peripheral blood of NOG mice engrafted with a NUP98-JARID1A PDX (CPCT0021) after 36 days of treatment with 0.1% VTP50469 or control chow. (n=3 mice per group.) One-way ANOVA was performed; bars represent mean + S.E.M. ****P<0.0001.

Supplemental Figure 7: (**A**) Survival analysis of NOG mice engrafted with a NUP98-NSD1 PDX (MSKG5191) treated with control or 0.1% VTP50469 chow. (**B**) Percent human CD45+ cells in the peripheral blood of NOG mice engrafted with the NUP98-NSD1 PDX as described above. (**C**) Disease burden in bone marrow and spleen at the time of sacrifice. n=5 mice per group; Box plots represent min to max; ns=not significant.

Supplemental Figure 8: (**A**) PCR using excision primers to confirm Mx1 Cre-mediated rearrangement at the *Wt1* locus: Lane 1, LSK cells from C57BL/6 mouse; Lanes 2-5, LSK cells from Wt1 flox/flox Mx1-Cre mice that received no treatment (2) or induced deletion of floxed alleles using pIpC (3-5). Lane 3 shows LSK cells that were also transduced with the NUP98-NSD1 fusion protein. See supplemental methods for excision primer sequences. (**B**) Quantitative PCR to confirm loss of *Wt1* gene expression in NUP98-NSD1 *Wt1*-/- mouse LSK cells. *Wt1* expression in NUP98-HOXA9 mouse is shown as a control. *Wt1* expression is normalized to *Gapdh* expression. (**C**)

NUP98-NSD1 *Wt1*-/- mouse leukemia cells treated with an escalating dose curve of VTP50469. Data are representative of 4 independent experiments. (**D**) Expression of CD11b on NUP98-NSD1 *Wt1*-/- mouse leukemia cells treated with an escalating dose curve of VTP50469. Data are representative of 4 independent experiments. Bars represent mean ± SEM. One-way ANOVA with Dunnett's multiple comparisons test was performed, ****P<0.0001. (**E**) Cytological preparations of NUP98-NSD1 *Wt1*-/- mouse leukemia cells treated *in vitro* with 200 nM VTP50469 for 9 days. Data are representative of 2 independent experiments. (**F**) Volcano plot depicting gene expression changes at day 3 of treatment with 200 nM VTP50469 in NUP98-NSD1 *Wt1*-/- mouse leukemia cells. n=3 replicates per group. (**G**) Quantitative PCR for *Meis1* at 24, 72, or 96 hours of treatment with 200 nM VTP50469 in NUP98-NSD1 *Wt1*-/- mouse leukemia cells Bars represent mean ± SEM. Two-way ANOVA with Dunnett's multiple comparisons test was performed, ***P=0.001, ****P<0.0001. (**H**) ChIPseq gene density plots showing changes in chromatin occupancy of Mll1 in reads per million (rpm) upon treatment with 200 nM VTP50469 in NUP98-NSD1 *Wt1*-/- mouse leukemia cells. Data are representative of 2 independent experiments.

Supplemental Figure 9: (**A**) Volcano plot depicting gene expression changes in human CD45+ cells isolated from NOG mice engrafted with a NUP98-NSD1 PDX (NTPL-511) and treated with control or 0.1% VTP50469 chow for 14 days. n=3 control mice, n=6 VTP50469 treated mice. (**B**) Quantitative PCR for *MEIS1, HOXA9, HOXA11, and MEF2C* in human CD45+ cells isolated from NOG mice bearing the NTPL-511 PDX model isolated after 14 days of treatment with VTP50469. Two-way ANOVA with

Dunnett's multiple comparisons test was performed, ****P<0.0001. n=3 control mice, n=6 VTP50469 treated mice. (**C-D**) Gene-set enrichment analysis (GSEA) of gene expression changes measured by RNAseq in human CD45 cells isolated from NUP98-NSD1 PDX bearing mice. (**C**) Top panels depict enrichment in gene sets among downregulated targets, and (**D**) bottom panels depict enrichment in gene sets among upregulated targets.

Supplemental Figure 10: (**A**) Volcano plot depicting gene expression changes in human CD45+ cells isolated from NOG mice engrafted with a NUP98-JARID1A PDX (CPCT0021) and treated with control or 0.1% VTP50469 chow for 36 days. n=3 mice per group. (**B-C**) Gene-set enrichment analysis (GSEA) of gene expression changes measured by RNAseq in human CD45 cells isolated from NUP98-NSD1 PDX bearing mice. (**B**) Top panels depict enrichment in gene sets among downregulated targets, and (**C**) bottom panels depict enrichment in gene sets among upregulated targets. (**D**) Immunohistochemistry staining for von Willebrand Factor (DAB chromogen) in femurs from control and treated NOG mice transplanted with a NUP98-JARID1A PDX, after 36 days of treatment with 0.1% VTP50469 chow. Images were acquired using an Olympus BX41 microscope using the 20X or 100X objective with acquisition software SPOT Version 5.1.

Supplemental Methods

Cell Lines. HEK293-T cells were acquired from American Type Culture Collection (ATCC) and maintained in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin (Gibco). Mouse leukemia cell lines were maintained in IMDM supplemented with 10% fetal bovine serum, 1x penicillin/streptomycin (Gibco), 10 ng/mL mouse IL-3, 10 ng/mL mouse IL-6, and 10 ng/mL mouse SCF (Stem Cell Technologies). All cells were maintained at 37C and 5% CO_2.

Cell Viability and Differentiation and Assays. Cells were treated with VTP50469 as indicated or 0.1% DMSO. The cells were re-plated every 3 days to maintain the initial density. Viable (DAPI-) cells were counted in 50 microliters of media using FACS Fortessa (BD Biosciences). Ratios of cell numbers were plotted to calculate IC_{50}. Differentiation status of VTP50469 or DMSO treated cells was assessed at day 9 by flow cytometry with CD11b-APC (BioLegend) and by cytospin and staining with hematoxylin and eosin. For Annexin and propidium iodide staining, the ratio of live, dead, and apoptotic cells was quantified using flow cytometry and staining using the eBioscience Annexin V Apoptosis Detection Kit APC (Life Technologies 88-8007-74).

Real-time quantitative PCR RNA was isolated using the RNeasy Mini Kit (Qiagen) and was reverse transcribed into cDNA using the AzuraFlex cDNA Synthesis Kit (Azura Genomics AZ-1997). Taqman gene expression master mix (Life Technologies 4369016) was used to perform real-time quantitative PCR for all samples in triplicate using the ViiA 7 Real-Time PCR system (Applied Biosystems Grand Island, NY). Fold change

relative to the control condition was calculated by the comparative ΔΔ cycle threshold method using *Gapdh* as the housekeeping gene for normalization. Mouse probes used: *Gapdh* (Mm99999915_g1), *Meis1* (Mm00487664_m1), *Wt1* (Mm01337048-mouse). Human probes used: *MEIS1* (Hs00180020_m1), *HOXA9* (Hs00266821_m1), *HOXA11* (Hs00194149_m1), *MEF2C* (Hs00231149_m1), and *GAPDH* (Hs02786624_g1).

Immunoblotting Protein gel electrophoresis was performed using 10% Tris-Acetate gels (ThermoFisher). Proteins were transferred onto nitrocellulose membranes using the iBlot2 gel transfer system semi-dry transfer method (Thermo Fisher IB21001). Membranes were blocked in 5% milk in TBS-Tween (TBST) for 1 hour and then incubated overnight with anti-Meis1 (Thermo Fisher MA527191), anti-Menin (Bethyl A300-105A) or Beta-Actin HRP conjugated (Cell Signaling 12620S). The following day, membranes were washed in TBST and developed directly (for Beta-Actin) or using a secondary rabbit anti-HRP (Cell Signaling 12620S) and chemiluminescence kit (Pierce).

Immunohistochemistry. Samples of BM, spleens and livers were fixed in 10% formalin and embedded in paraffin for sectioning. Sections were stained with Haemotoxylin and Eosin (H&E) to visualize hematopoietic cells. Immunohistochemistry was performed on the Leica Bond III automated staining platform. Polyclonal antibody for human von Willebrand Factor (VWF; Agilent Dako A0082) was performed at 1:1000 dilution with citrate antigen retrieval using the Leica Biosystems Refine Detection kit.

Isolation of LSK Cells. For generation of NUP98 rearranged mouse leukemia cells, hematopoietic progenitors were isolated from the bone marrow of 8-10 week old

C57BL/6 mice or *Mx1*-Cre *Wt1 flox/flox* mice by crushing tibiae, femurs, pelvis and vertebrae. LSK cells were enriched from bone marrow using the EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit (StemCell Technologies 19856). Enriched cells were stained with lineage markers using anti-mouse Lineage Cocktail-Pacific Blue (BioLegend) and were isolated by positive selection using flow sorting for the following markers: anti-mouse Sca1-Alexa Fluor 647 and anti-mouse CD117-APC (BioLegend). Prior to retroviral transduction to introduce NUP98 fusion proteins, cells were cultured for 24 hours in StemSpan SFEM media (StemCell Technologies) containing the following recombinant murine cytokines: 50 ng/ml Flt3, 50 ng/ml thrombopoietin (TPO), and 50 ng/ml stem cell factor (mSCF) (StemCell Technologies).

Retroviral transduction of LSK Cells with NUP98 fusions. NUP98-HOXA9 and NUP98-JARID1A[28] were cloned into pMSCV-IRES-GFP or pMSCV-IRES-TdTomato (Addgene 20672, 97079) containing either IRES-GFP or IRES-TdTomato, respectively. Each fusion was tagged at the C-terminus with FKBP12 FV36 and HA. For biotinylated NUP98 fusion protein experiments, NUP98-HOXA9 and NUP98-HOXD13 mouse leukemia cells were generated as previously described[13]. Retroviral supernatants capable of transducing murine cells were generated by co-transfection with the NUP98-fusion containing plasmids described above, in addition to the pCL Eco viral packaging vector (Addgene 12371). Viral supernatants were harvested at approximately 40 hours after transfection and were concentrated using Amicon columns (Thermo Scientific UFC903024). LSK cells were spin infected by centrifugation at 2000 rpm at 37 °C for 1.5 hours. Polybrene was added to a final concentration of 1.0 ug/ml. Following spin

infection, media containing viral supernatant was replaced with fresh IMDM containing the following cytokines: 10 ng/mL mouse IL-3, 10 ng/mL mouse IL-6, and 10 ng/mL mouse SCF (Stem Cell Technologies). To isolate cells that had been transformed by NUP98 fusion proteins, cells were sorted for GFP (NUP98-HOXA9) or TdTomato (NUP98-JARID1A).

PCR to confirm loss of Wt1. Excision of floxed Wt1 alleles was confirmed using Wt1 Excision-Primer 1.55: 5' TGCCTACCCAATGCTCATTG 3' and Wt1 Excision F Primer: 5' GCTAACATATGGGAGACATT 3' as previously described[19-20]. Deletion of floxed alleles resulted in a 342 bp PCR product.

In vitro CRISPR Competition Assays. Stable Cas9 expressing cell lines were generated by introducing pLentiCas9-vBlast (Addgene 52962) into NUP98-HOXA9 or NUP98-JARID1A mouse leukemia cells using the lentiviral packaging vectors pMD2G (Addgene 12259) and psPax2 (Addgene 12260). Clones were isolated by picking single colonies from methylcellulose and were tested for their CRISPR editing efficiency. Small guide RNAs were designed using the Zhang laboratory's CRISPR design tool34 and were cloned into an improved scaffold pU6-sgRNA- EF1Alpha-PURO-T2A-RFP (ipUSEPR) vector or pLKO5.sgRNA.EFS.GFP (Addgene 57822). Sequences of small guide RNA used in these experiments appear in supplemental Table 1. Lentivirus was produced by transfecting the plasmids described above in HEK293-T, then by harvesting and concentrating viral supernatants as described above. Virus was titered to an MOI of 0.2-0.3, such that approximately 20-30% of cells would be RFP+ or GFP+.

Within each well of a 96-well plate, the ratio of live (DAPI-) fluorescent marker positive to negative cells was quantified at each timepoint. For confirmation of CRISPR editing in NUP98-HOXA9 cells, RFP+ cells harboring sgRNA were sorted on BD FACSAria II. Genomic DNA was isolated from sorted cells and PCR amplicons were generated for next-generation sequencing using primers flanking the CRISPR cut site. In addition, RFP+ sorted cells were lysed and subjected to electrophoresis and immunoblotting to detect Menin protein in cells that had undergone CRISPR editing.

CRISPR-mediated activation of Meis1. Stable dead Cas9 (dCas9) cell lines were generated by introducing pLenti dCAS-VP64_Blast (Addgene 61425) into mouse NUP98-HOXA9 or NUP98-JARID1A leukemia cells. The sgRNA (MS2) cloning backbone (Addgene 61424) was used to generate clones which stably express sgRNA targeting *Meis1* or the luciferase control. Sequences of small guide RNA used in these experiments appear in **supplemental Table 2**. Clones were isolated by picking single colonies from methylcellulose and were tested for their ability to activate Meis1 transcription by quantitative real time PCR and by immunoblotting for Meis1.

Meis1 overexpression. Stable cell lines overexpressing Meis1 or an empty vector control were generated by retroviral transduction of NUP98-HOXA9 or NUP98-JARID1A mouse leukemia cells as previously described[18].

RNA-Seq Assays. For in vitro RNA-seq experiments, the cells were treated with VTP50469 (concentrations as indicated in figure legends) or DMSO for the indicated time. For *in vivo* RNA-seq experiments, PDX cells were harvested from the BM of

vehicle control treated mice at 14 or 36 days of treatment. Human cells were enriched from the bone marrow of mice using the mouse cell depletion kit (Miltenyi Biotec (valid) # 130-104-694). RNA was extracted using the RNeasy kit (Qiagen). RNA (1 ug) was used to make Illumina compatible 3'-end libraries using QuantSeq 3'mRNA kit (Lexogen, Catalog numbers E7490L and E7530L). RNA-seq libraries were then sequenced on NextSeq550 (Illumina) to obtain 20-25 million unique sequencing reads.

Quantification and Statistical Analyses Statistical analysis was performed using Microsoft Excel and GraphPad Prism software (v.9.0.2) were used for statistical analysis. Bars represent mean +/- standard error of the mean, and a p-value <0.05 was considered significant. Analysis of variance (ANOVA) was used to calculate statistical significance with Dunnett's post-test to correct for multiple comparisons. For in vivo leukemia experiments, the Kaplan-Meier method was used to plot survival and the log-rank test was used to determine statistical significance.

Next generation sequencing. Illumina sequencing compatible libraries for RNAseq were generated as above, and libraries for ChIPseq were generated using the SMARTer ThruPLEX DNA-Seq Kit (Takara). Library size was analyzed by Tapestation (Agilent) and library concentration was quantified using KAPAquant (Roche diagnostics 07960140001). The NextSeq® 500 High Output v2 Kit (75 cycles) (Illumina 20024906) was used for sequencing on an Illumina NextSeq500 platform.

Data Analysis. Raw Illumina sequencer output was converted to FASTQ format using bcl2fastq (v2.20.0.422). Reads (paired-end 37-mers) were aligned to the human (Gencode GRCh38/hg38 v33) or mouse (Gencode M24/mm10) genome using STAR

(v2.7.5a),sorted and duplicates marked/removed with picard pipeline tools (v2.9.4). Final “deduped” BAM files were indexed using SAMtools (v1.95). Data visualizations were produced using IGVtools (TDF signal pileups; v2.3.75). For RNAseq, raw per-gene counts were calculated with HTSeq (htseq-count, v0.6.1pl). Differential RNA-seq expression was calculated using the BioConductor DESeq2 package (v1.24,0), using raw unnormalized per-gene counts from deduplicated BAMs. Results were filtered to exclude non-protein- coding genes (based on Ensembl gene biotype annotations) and low-expressed genes (those with values less than 1 in DESeq2 rlog scale for both treatments). Expression differentials of greater than two-fold and adjusted pValue < 0.05 were considered significant. Counts were converted to counts per million reads (CPM) for each sample for further analysis. CPM values were used in gene set enrichment analysis (GSEA) to assess enrichment significance of tested gene sets in either DMSO or VTP50469 phenotypes. ChIPseq signal peaks were called using MACS2 (v2.1.4). MACS2 peak overlaps were determined using bedtools (intersect function; v2.28.0) and signal within peak regions and other defined genomic intervals was determined using bedtools (coverage). ChIP-seq data “tornado plot” heatmap visualizations were produced using ngs.plot.