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Supplementary Information for

Depletion of H3K36me2 recapitulates epigenomic and phenotypic changes induced by the H3.3K36M oncohistone mutation

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This PDF file includes:

Figures S1 to S3
Tables S1 to S3
Legends for Datasets S1 to S2

Other supplementary materials for this manuscript include the following:

Datasets S1 to S2

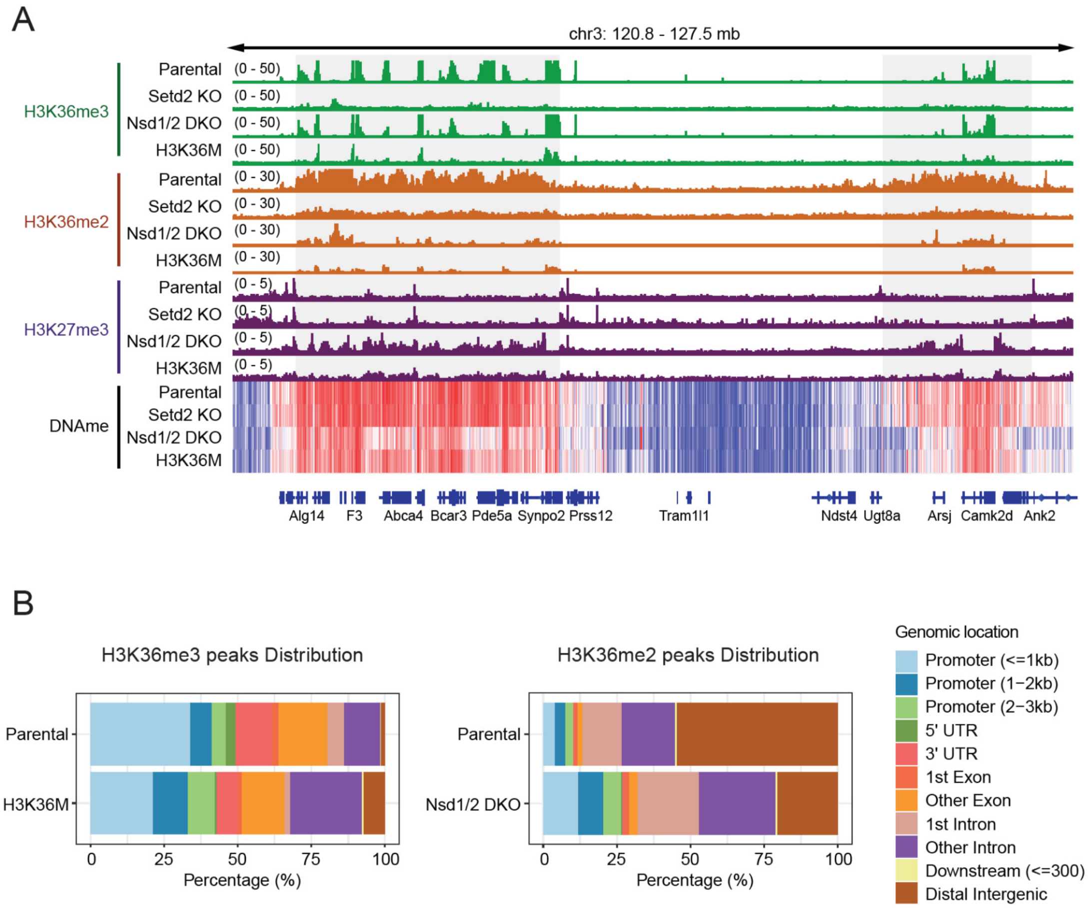

Figure S1: related to Figure 1.

(A) IGV snapshot showing the chromatin landscape of H3K36me3, H3K36me2, H3K27me3, and DNA methylation at a representative genomic region in parental, H3K36M, Nsd1/2 DKO and Setd2 KO 10T cells.

(B) Annotation of genomic distribution of (left) H3K36me3 peaks in Parental and H3K36M cells; (right) H3K36me2 peaks in Parental and Nsd1/2 DKO cells.

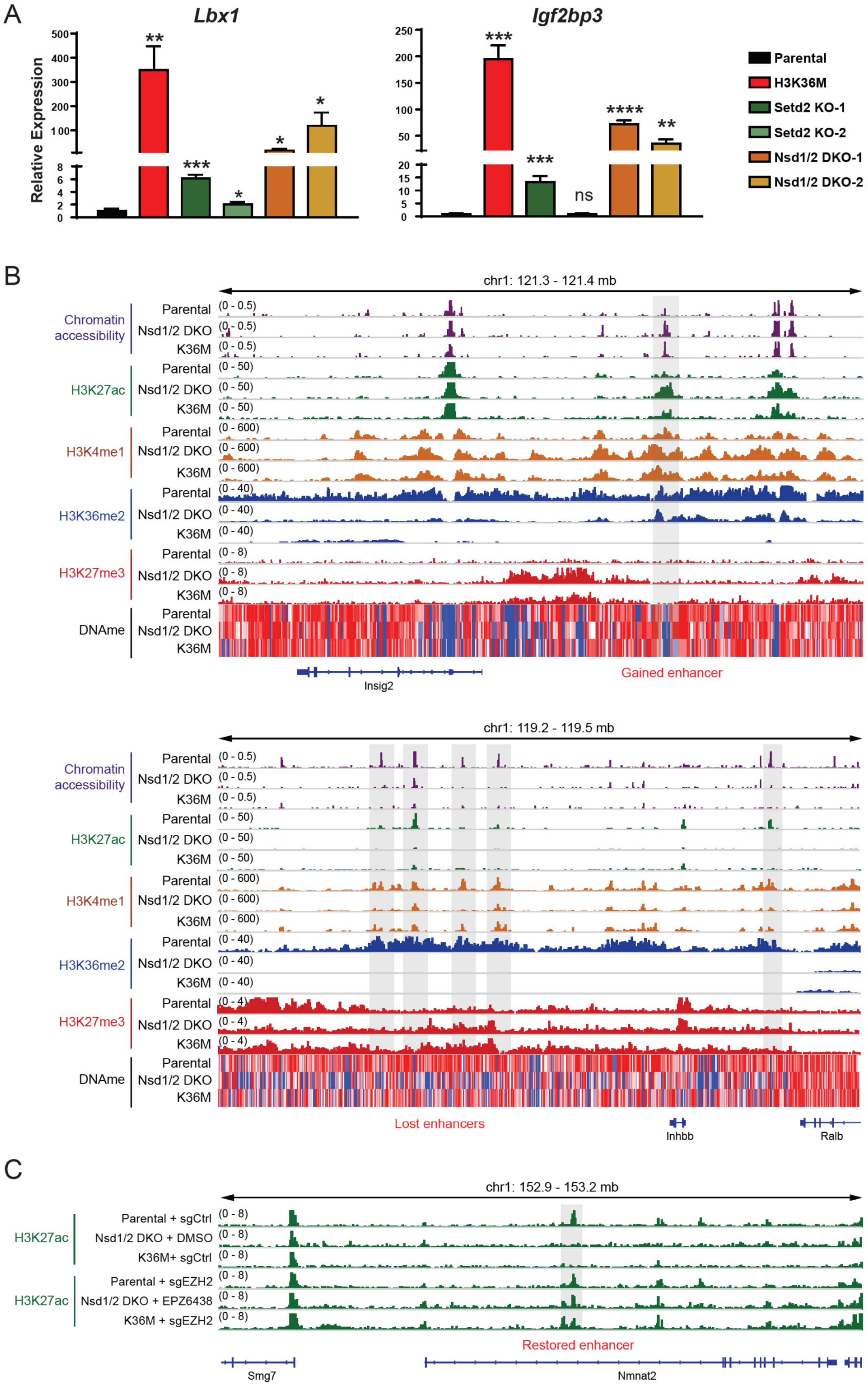
A
Lbx1
Relative Expression
500
400
300
200
100
8
6
4
2
0
**
***
*
*
*
Igf2bp3
250
200
150
100
50
20
15
10
5
0
***
***
ns
****
**
Parental
H3K36M
Setd2 KO-1
Setd2 KO-2
Nsd1/2 DKO-1
Nsd1/2 DKO-2
B
chr1: 121.3 - 121.4 mb
Chromatin accessibility
H3K27ac
H3K4me1
H3K36me2
H3K27me3
DNAme
Parental
Nsd1/2 DKO
K36M
(0 - 0.5)
(0 - 50)
(0 - 600)
(0 - 40)
(0 - 8)
Insig2
Gained enhancer
chr1: 119.2 - 119.5 mb
(0 - 4)
Lost enhancers
Inhbb
Ralb
C
chr1: 152.9 - 153.2 mb
H3K27ac
Parental + sgCtrl
Nsd1/2 DKO + DMSO
K36M+ sgCtrl
Parental + sgEZH2
Nsd1/2 DKO + EPZ6438
K36M + sgEZH2
(0 - 8)
Restored enhancer
Smg7
Nmnat2

Figure S2: related to Figure 3.

(A) Expression of two representative PRC1-silenced genes *Lbx1* and *Igf2bp3* as measured by RT-PCR in parental, H3K36M, Nsd1/2 DKO and Setd2 KO 10T cells. Error bars represent standard deviation from n=3. T-test was performed to compare all samples to parental cells. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; ****, $p<0.001$; ns, not significant.

(B) IGV snapshots showing the chromatin landscape of H3K36me2, H3K27ac, H3K27me3, H3K4me1, DNA methylation and ATAC-seq at representative genomic regions in parental, Nsd1/2 DKO and H3K36M cells. Enhancers that are gained or lost in Nsd1/2 DKO cells are indicated.

(C) IGV snapshots showing the chromatin landscape of H3K27ac at a representative genomic region in parental, Nsd1/2 DKO, and H3K36M cells with or without *Ezh2* knockout or EZH2 inhibitor EPZ-6438. An enhancer that is lost in Nsd1/2 DKO and H3K36M cells but restored upon *Ezh2* knockout or EPZ-6438 treatment is indicated.

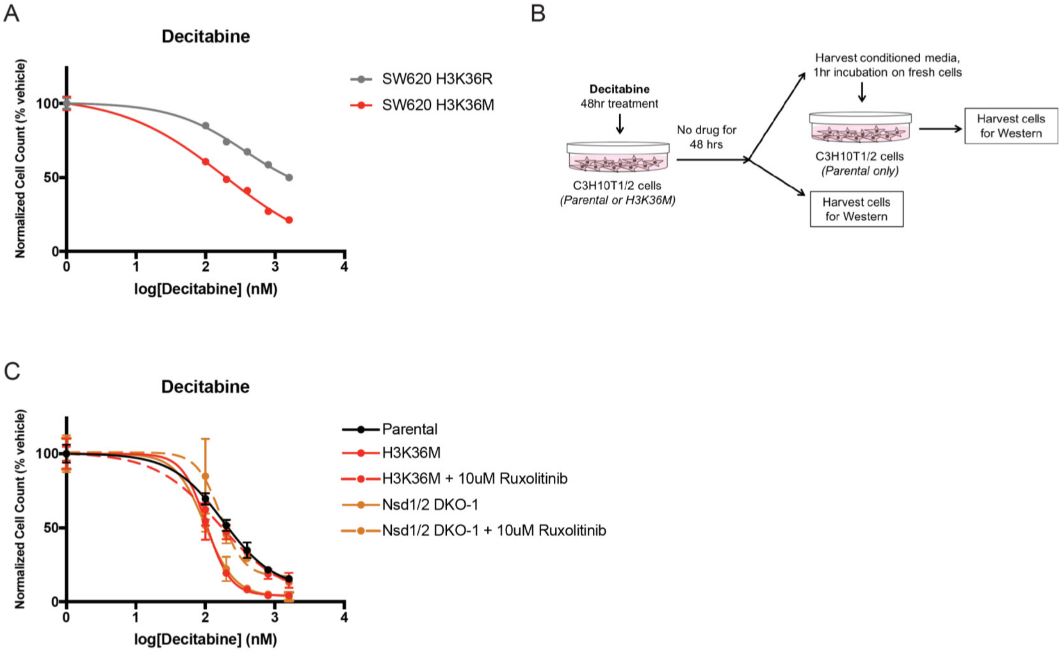

Figure S3: related to Figure 4.
(A) Normalized cell count of colorectal cancer cell line SW620 expressing H3.3K36M or H3.3K36R after treatment with increasing concentrations of decitabine. Two-way ANOVA was performed to compare all samples to parental cells and the *p*-values are listed in Table S2. n=3.
(B) Schematic for conditioned media treatment followed by Western blotting shown in Fig. 4E.
(C) Normalized cell count of parental, H3K36M and Nsd1/2 DKO cells after treatment with increasing concentrations of decitabine with or without the JAK1/2 inhibitor Ruxolitinib. Error bars represent standard deviation from n=3. Two-way ANOVA was performed to compare all samples to parental cells and the *p*-values are listed in Table S3.

Table S1: Drug Dose Responses in Parental, H3K36M, Setd2 KO-1, and Nsd1/2 DKO-1 Cells			
	Decitabine	**Cytarabine**	**Wee1 inhibitor**
Dose 1 (0 nm)			
Parental vs. H3K36M	ns	ns	ns
Parental vs. Setd KO-1	ns	ns	ns
Parental vs. Nsd1/2 DKO-1	ns	ns	ns
Dose 2			
Parental vs. H3K36M	****	**	****
Parental vs. Setd KO-1	****	****	****
Parental vs. Nsd1/2 DKO-1	****	ns	***
Dose 3			
Parental vs. H3K36M	****	ns	ns
Parental vs. Setd KO-1	****	****	****
Parental vs. Nsd1/2 DKO-1	****	ns	****
Dose 4			
Parental vs. H3K36M	****	ns	***
Parental vs. Setd KO-1	***	****	****
Parental vs. Nsd1/2 DKO-1	****	ns	****
Dose 5			
Parental vs. H3K36M	****	ns	***
Parental vs. Setd KO-1	**	****	****
Parental vs. Nsd1/2 DKO-1	****	ns	****
Dose 6			
Parental vs. H3K36M	****	ns	***
Parental vs. Setd KO-1	*	ns	****
Parental vs. Nsd1/2 DKO-1	****	ns	*
Dose 7			
Parental vs. H3K36M	N/A	N/A	****
Parental vs. Setd KO-1	N/A	N/A	ns
Parental vs. Nsd1/2 DKO-1	N/A	N/A	ns
Dose 8			
Parental vs. H3K36M	N/A	N/A	**
Parental vs. Setd KO-1	N/A	N/A	ns
Parental vs. Nsd1/2 DKO-1	N/A	N/A	ns

Table S1: related to Figure 4A-C.
Two-way ANOVA was performed to compare all samples to the parental cells. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; ****, $p<0.001$.

Table S2: Decitabine Dose Response in SW620 H3K36R vs. SW620 H3K36M	
Dose 1 (0 nm)	ns
Dose 2	****
Dose 3	****
Dose 4	****
Dose 5	****
Dose 6	****

Table S2: related to Figure S3A
Two-way ANOVA was performed to compare all samples. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; ****, $p<0.001$.

Table S3: Decitabine Dose Response in Parental, H3K36M, and NSD1/2DKO-1 Cells with Ruxolitinib	
Dose 1 (0 nm)	
Parental vs. H3K36M	ns
Parental vs. H3K36M + 10 uM Ruxolitinib	ns
Parental vs. Nsd1/2 DKO-1	ns
Parental vs. Nsd1/2 DKO-1 + 10 uM Ruxolitinib	ns
Dose 2	
Parental vs. H3K36M	****
Parental vs. H3K36M + 10 uM Ruxolitinib	*
Parental vs. Nsd1/2 DKO-1	****
Parental vs. Nsd1/2 DKO-1 + 10 uM Ruxolitinib	****
Dose 3	
Parental vs. H3K36M	****
Parental vs. H3K36M + 10 uM Ruxolitinib	ns
Parental vs. Nsd1/2 DKO-1	****
Parental vs. Nsd1/2 DKO-1 + 10 uM Ruxolitinib	*
Dose 4	
Parental vs. H3K36M	****
Parental vs. H3K36M + 10 uM Ruxolitinib	ns
Parental vs. Nsd1/2 DKO-1	****
Parental vs. Nsd1/2 DKO-1 + 10 uM Ruxolitinib	ns
Dose 5	
Parental vs. H3K36M	****
Parental vs. H3K36M + 10 uM Ruxolitinib	ns
Parental vs. Nsd1/2 DKO-1	****
Parental vs. Nsd1/2 DKO-1 + 10 uM Ruxolitinib	ns
Dose 6	
Parental vs. H3K36M	****
Parental vs. H3K36M + 10 uM Ruxolitinib	ns
Parental vs. Nsd1/2 DKO-1	****
Parental vs. Nsd1/2 DKO-1 + 10 uM Ruxolitinib	ns

Table S3: related to Figure S3C
Two-way ANOVA was performed to compare all samples to the parental cells. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; ****, p<0.001.

Dataset S1 (separate file). Annotation of H3K36me3 peaks that remain in H3K36M 10T cells, related to Figure 1.

Dataset S2 (separate file). Lists of differentially expressed genes between H3K36M and Nsd1/2 DKO 10T cells and the most affected pathways, related to Figure 2. Thresholds for differentially expressed genes: fold change > 2, FDR < 0.1.