**Reynolds et al. Supplementary Information**

**Supplementary Figures**

**Supplementary Figure 1. ChIP for NuRD component proteins.**

ChIP-qPCR for putative NuRD targets for proteins other than Mi2β which are unique to NuRD (Mbd3, MTA2) or which are not uniquely found in NuRD (HDAC1). qPCR is shown relative to level in IgG control either at regions close to TSS for individual genes, or across a single target locus, *Htra1*. Distance from TSS is shown in base pairs.

**Supplementary Figure 2. Distribution of histone modifications.** (A) At the whole gene level. Distance is shown in base pairs for upstream, 3kb Metagene, and downstream regions. (B) At transcription termination sites. Profiles, normalized to input, are of up-regulated, bivalent genes for αH3K27me3 and αH3K27ac ChIP samples (solid line) relative to that for all RefSeq genes in each sample (dotted line).

**Supplementary Figure 3. Distribution of histone modifications for genes bound by Mi2β.** Profiles, normalized to input for genes bound by Mi2β at transcription termination site (TSS) for αH3K27me3 and αH3K27ac ChIP samples. Profiles shown are for genes bound by Mi2β (solid line) relative to that for all Ref-Seq genes in each sample (dotted line).

**Supplementary Figure 4. NuRD and PRC2 complexes do not co-immunoprecipitate in ES cells.** (A) Immunoprecipitation of Mi2β or IgG control probed for presence of Jarid2, MTA2 and Mi2β. (B) Avi-tagged Mbd3 pulled down using streptavidin beads,

probed for presence of PRC2 component Ezh2 or known NuRD interactor Sall4. Pull-downs were performed in the presence or absence of nucleases as indicated. (C) Immunoprecipitation of either MTA2 or Jarid2, probed for the presence of Suz12 or MTA2. All precipitations were carried out using nuclear extracts from wild type ES cells.

**Supplementary Figure 5. Effect of TSA treatment on transcription over time.** Quantitative RT-PCR from cells grown in TSA for varying times, or from *Mbd3*$^{-/-}$ cells shown relative to wild type expression levels in the absence of TSA. Error bars indicate sem.

**Supplementary Figure 6. Distribution of histone modifications for genes at which PRC2 occupancy is dependent on NuRD.** Profiles, normalized to input at transcription start site (TSS) for αH3K27me3 and αH3K27ac ChIP samples at those genes bound by Suz12 in wt cells but not in *Mbd3*$^{-/-}$ and which are also bound by Mi2β (solid line) relative to that for all Ref-Seq genes in each sample (dotted line).

**Supplementary Table 1. Results of microarray analysis comparing *Mbd3*$^{-/-}$ to wild type cell lines.**

**Supplementary Table 2. Summary of genomic regions bound by Mi2β in wild type ES cells.**

**Supplementary Table 3. Summary of ChIP-Seq data for H3K27 modifications in wild type and *Mbd3*$^{-/-}$ ES cells.**

**Supplementary Table 4. Comparison of gene expression patterns in Mbd3-null and PRC-null ES cells.**

**Supplementary Table S5. Summary of genomic regions bound by Suz12 in wild type and *Mbd3*$^{-/-}$ ES cells.**

**Supplementary Table 6a. Primers For Expression Analysis**

| Gene | Forward | Reverse |
|---|---|---|
| *β Actin* | GTGGGCCGCTCTAGACACCA | CGGTTGGCCTTAGGGTTCAGGGGGG |
| *Htra1* | ACTTCGGAACTCCGATATGG | CGTGGGACTCTGTCAAGAAC |
| *Klf2* | CTAAAGGCGCATCTGCGTA | TAGTGGCGGGTAAGCTCGT |
| *Klf4* | CGGGAAGGGAGAAGACACT | GAGTTCCTCACGCCAACG |
| *Klf5* | CCGGAGACGATCTGAAACAC | CAGATACTTCTCCATTTCACATCTTG |
| *Lefty2* | GCAGGTCCAGGTACATCTCC | ACACGCTGGACCTCAAGGAC |
| *Mcm6* | GAGAAACACGCTGGTTGTGA | AAGGTCTTCAAGGCTCGACA |
| *Pp1A* | CACGGGGGCCTGTCTCCAGA | GTCAGGCACGTCTGTGGGCC |
| *Ppp2r2c* | TTCCCGCTGGAAGATAACC | CGCGGAAAATTAACCACAGC |
| *Smad7* | TCTCCCCCTCCTCCTTACTC | TCCAGAAGAAGTTGGGAATC |
| *Sohlh2* | TTCTGATTTGTCCTGGCAGC | TATTCCATGACTGCTGCAGG |
| *Sox9* | CCACGGAACAGACTCACATCTCTC | CTGCTCAGTTCACCGATGTCCACG |
| *T* | TGCTTCCCTGAGACCCAGTT | GATCACTTCTTTCCTTTGCATCAAG |
| *Tbx3* | GAACCTACCTGTTCCCGGAAA | CCATTGCCAGTGTCTCGAAAAC |

**Supplementary Table 6b. Primers For Chromatin IP Analysis**

| Gene | Forward | Reverse | Distance of mid-point |
|---|---|---|---|

| | | | from TSS (bp) |
|---|---|---|---|
| *β Actin* | GCCTAGTAACCGAGACATTGA | AGAAAGCGAGATTGAGGAAG | -3250 |
| *Cdx2* | AAGCCTGCCTTTCTGGACTT | TACGAGCTTCCTCCTTCCAA | -280 |
| *Htra1* | AGCAGCACCCTTGATCCTAA | GGGATGCCAGACAGAAAGAA | -2040 |
| *Htra1* | TGGCGAGGTGCATGGGGAACT | AGTCCCAGCGCTCGGGCAAA | -696 |
| *Htra1* | TCCCAGCGCTCGGGCAAATC | TCTGCCTCCGGGGTGACAGT | -646 |
| *Htra1* | ACTTGAAACTAGGTCTGGGC | GGCACTTAACAGAGGGAAAC | -236 |
| *Htra1* | GGTTTCCCTCTGTTAAGTGC | GGCTCAGTTTCTCATTCTAGG | -109 |
| *Htra1* | GTCACCGCCGCTAGGCCAATG | GGGTCTTGGGGACAGCGGGT | 65 |
| *Htra1* | GCGGCTCCTTCCTTGGCGTT | TGGGATCGCAGTGCTCGGGA | 250 |
| *Htra1* | CTCGCCAACTCAGCCCGACC | TGCTTCTCTGCACCTCCGCA | 708 |
| *Htra1* | GGGTGTTCTAGGCATCCAGT | GATGCTTGGCGCAGAAATAG | 795 |
| *Htra1* | GAGGGGCCCACTGGGGATGAA | TGTCGCCACCACCACGTCCA | 1356 |
| *Htra1* | GGCCCCAGTGGCCCCTAAGT | AGTCAGGGCGAAGCCAGGCT | 2733 |
| *Htra1* | TCTGCTAACAGTGCCAATGC | ACAGGTTCACGATCCACACA | 3643 |
| *Klf2* | GGGGCTTGAGGCTGGGGAGA | GGCGACGGCGTCAACAAACC | -265 |
| *Klf4* | CTCACCCCCACCCTACG | ATTATCCGCGTGACTCATCC | -570 |
| *Klf5* | GTCGGAGGCGGGACCTCGTG | GTCGGAGGCGGGACCTCGTG | -66 |
| *Lefty2* | GAACACACATCAGGTGGTGG | CAGGGCAGACTTCTTTGAGG | -4500 |
| *Mcm6* (Peng et al, 2009) | CTTATCGGAGGCACCTATAGTGAT | CTAACTCTCCTAGCCTCCTGACAC | 993 |
| *Ppp2r2c* | CCGCGATATCCTCGCGCTCC | AGCGGTCACCTCCGGAACCA | -186 |
| *Smad7* | GGTGGCAGTAACTGGGAGG | CGTCTAGACACCCTGTCGCT | -397 |

| | | | |
|---|---|---|---|
| *Sohlh2* | CCATTGGTTCTCAAGTCAGC | GGCGGTTTCTTTAATTCAGGAT | -328 |
| *Sox9*[1] | TATTAGAGACCCTGAGCTGGAAGT | CTGGACTGAAACTGGTAAAGTTGT | 24 |
| *T* | GGGAGTGGGGACTGCCCGAA | GTGCCAGGGAATGACGGGCC | -3265 |
| *T* | GCGGAGGCTCAGGCACGAAG | CTGGGCACTGCTGGCTGCTT | -2117 |
| *T* | GGTCGGGGTTGGGCGCAAATG | AGCCAACCTCTGGGGTGGGATG | -1399 |
| *T* | CAGTCCATGGGGCGAGGGGA | GGCGTCTCCCGGGTCTCCTT | -629 |
| *T* | CTGCGCCCGACGCTTTCCTTA | CTCCCGCAAGGCGCGACAAG | -234 |
| *T* | TCTCGGTGCTCCTTTGGCGAAT | CGCTGAGCAGGTGGTCCACTC | 64 |
| *T* | CCTACCTGCCGTTCTTGGTCACA | GTGGGCCTGGAGGAGAGCGA | 278 |
| *T* | CACCGAACGCGAACTGCGAG | ACAAAGTCGGCCGGTTGGGAA | 312 |
| *T* | CTTTGTTTCTTCCCGCTGAG | GCAAACCTGGTCATTCCAGT | 474 |
| *T* | CTGGCTCTGGCCATAGGTGAGC | GTGCAGAGTAGCCAGTGTCCCCT | 1311 |
| *T* | GCCCGCAACGCATGATCAC | GGGACTCCCTTCCGAAACCTCAC | 1796 |
| *T* | ACGGGAAAGTCCTGGCAAAGGCT | GCCCCAGGGGACTGATGACACA | 2113 |
| *Tbx3* | AGGCACCCAGAGATAAGTGTGATG | GAGCTGCCGCTCTGGGCTTG | -817 |

**Supplementary Table 7**

**Antibodies**

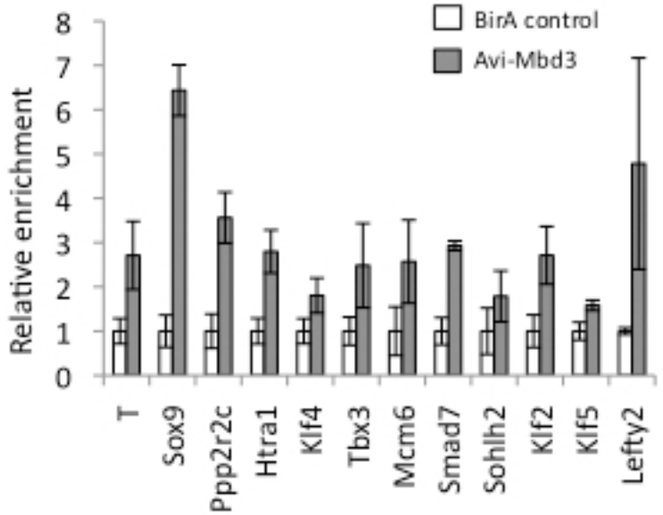
| Antibody | Source | Reference |
|---|---|---|
| α Mi2β | Abcam | ab70469 |
| α MTA2 | Santa Cruz | sc-9447 |
| α Mbd3 | Santa Cruz | sc-9402 |
| α Suz12 | Cell Signalling | 3737s |
| α Ezh2 | Active Motif | 39104 |
| α Eed | Millipore | 09-774 |

| | | |
|---|---|---|
| α Jarid2 | Novus Biologicals | NB100-2214 |
| α H3K4me3 | Millipore | 04-745 |
| α H3K9ac | Millipore | 06-942 |
| α H3K9me3 | Abcam | ab8898 |
| α H3K14ac | L. O'Neill, University of Birmingham, UK. | |
| α H3K36ac | Millipore | 07-540 |
| α H4K16ac | L. O'Neill, University of Birmingham, UK. | |
| α H4K5ac | L. O'Neill, University of Birmingham, UK. | |
| α H3K27ac | Abcam | ab4729-25 |
| α H3K27me3 | Millipore | 07-449 |
| α H3 | Abcam | ab1791 |
| α tubulin | Santa Cruz | sc-5286 |
| α ERα | Santa Cruz | Sc-543 |
| Rabbit IgG from serum | Sigma | I8140 |
| Mouse IgG from serum | Sigma | I8765 |

**Supplementary References**

Leeb M, Pasini D, Novatchkova M, Jaritz M, Helin K, Wutz A (2010) Polycomb complexes act redundantly to repress genomic repeats and genes. *Genes & development* **24:** 265-276

Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, Wysocka J (2009) Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* **139:** 1290-1302


BirA control
Avi-Mbd3
Relative enrichment
0
1
2
3
4
5
6
7
8
T
Sox9
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Htra1
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Tbx3
Mcm6
Smad7
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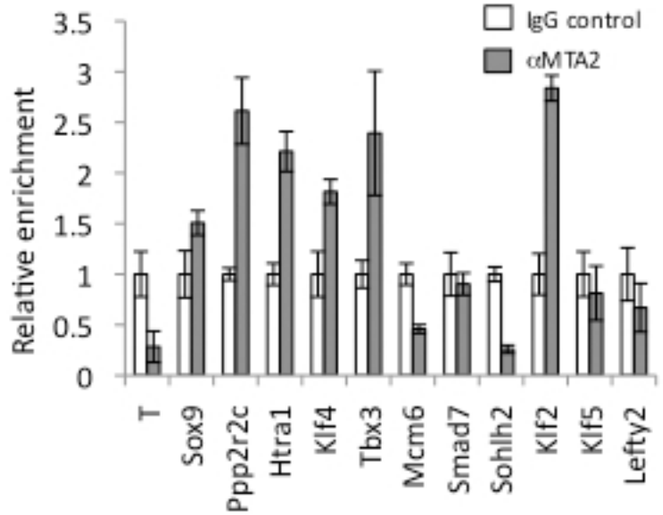
Klf5
Lefty2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αMTA2
Relative enrichment
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1
1.5
2
2.5
3
3.5
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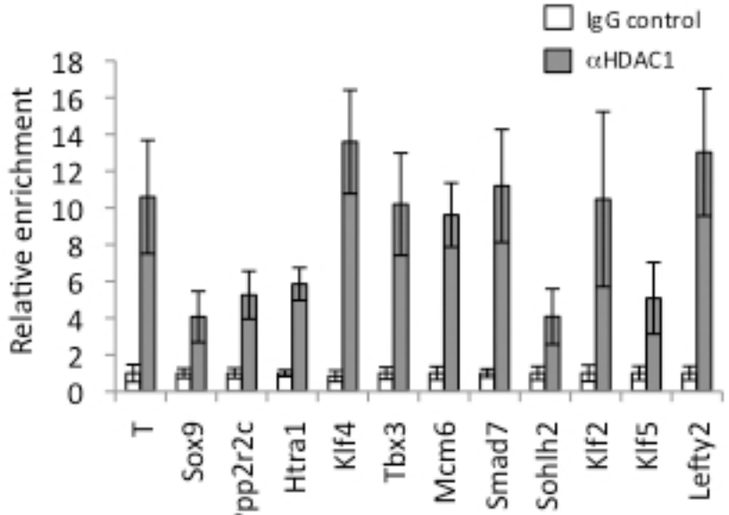
Klf4
Tbx3
Mcm6
Smad7
Sohlh2
Klf2
Klf5
Lefty2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Relative enrichment
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16
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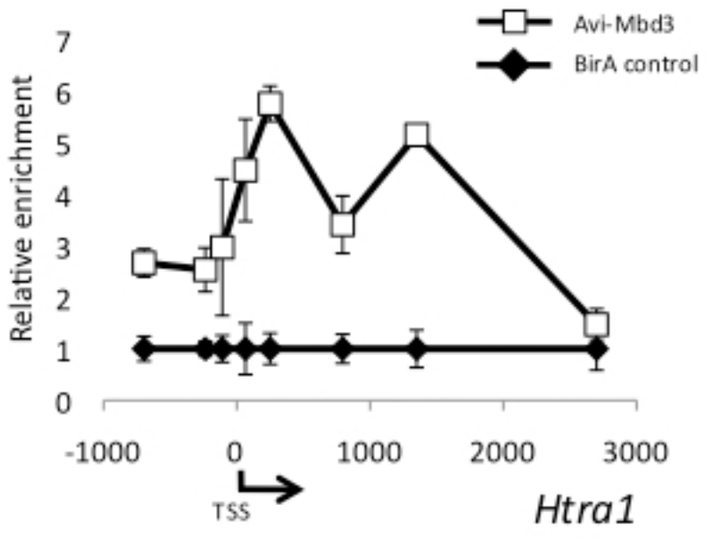
Avi-Mbd3
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Relative enrichment
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5
6
7
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0
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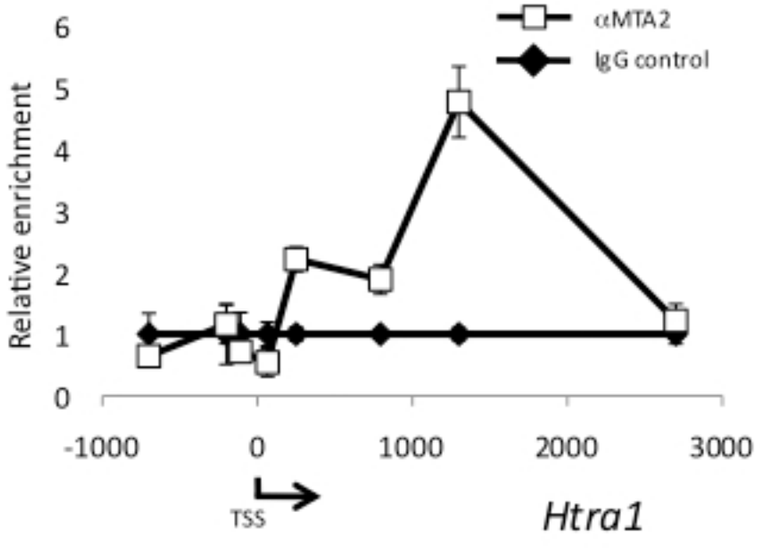
2000
3000
TSS
Htra1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IgG control
Relative enrichment
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1000
2000
3000
TSS
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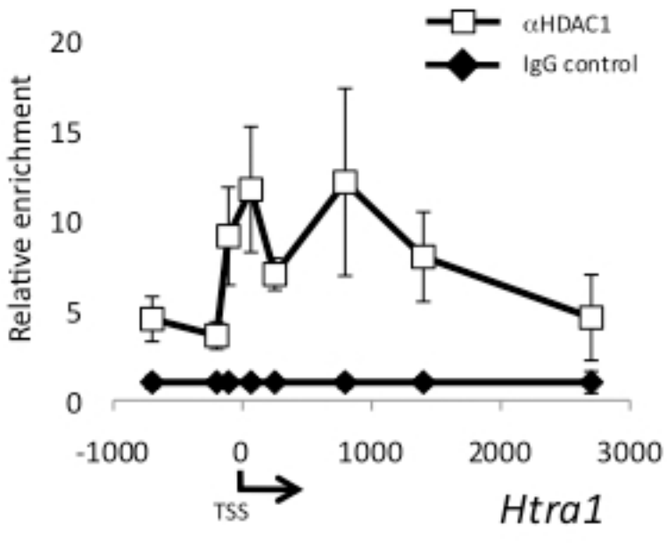
αHDAC1
IgG control
Relative enrichment
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5
10
15
20
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1000
2000
3000
TSS
Htra1

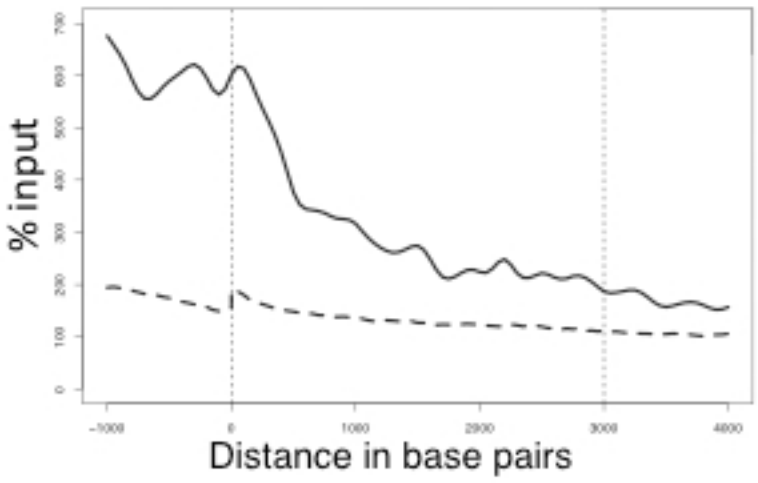
Reynolds_Supp Figure 2

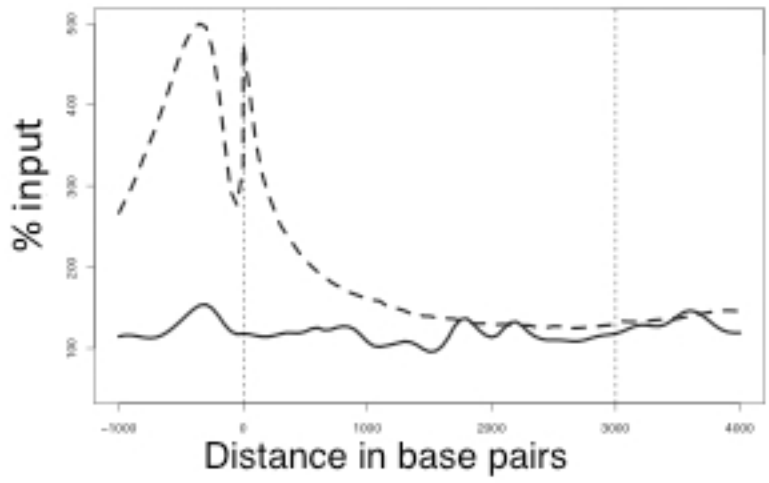
A

αH3K27me3

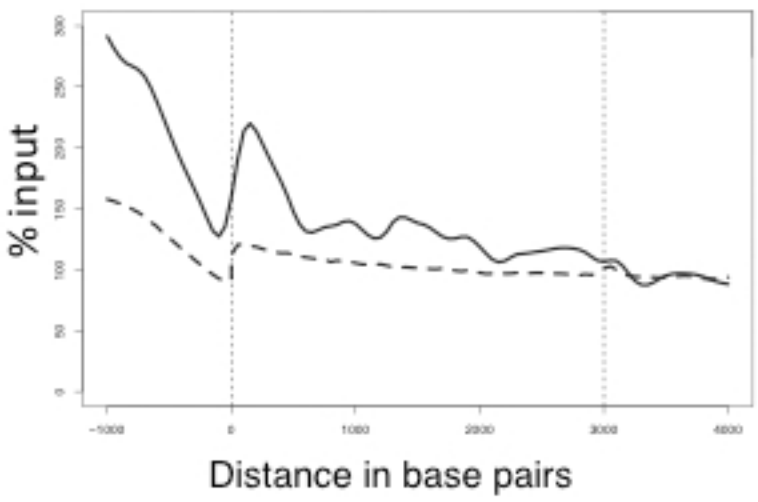
αH3K27ac

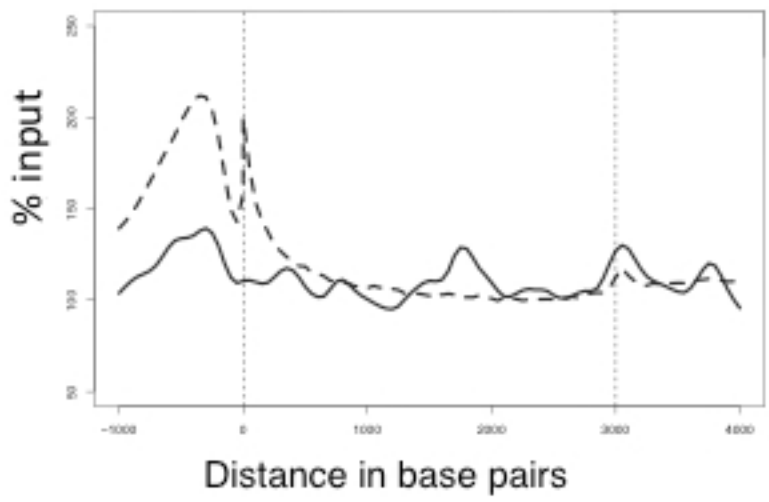
Wild Type

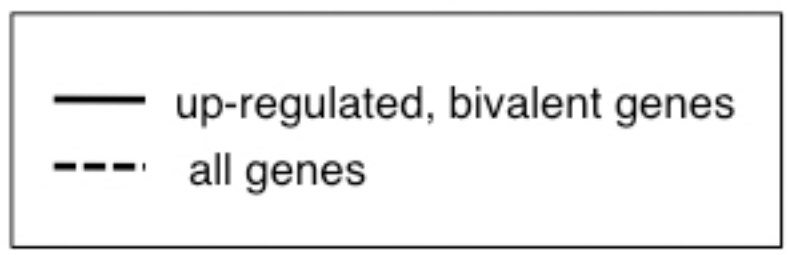



*Mbd3*$^{-/-}$



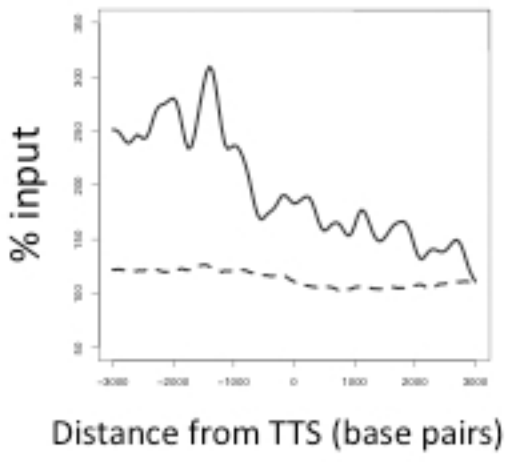



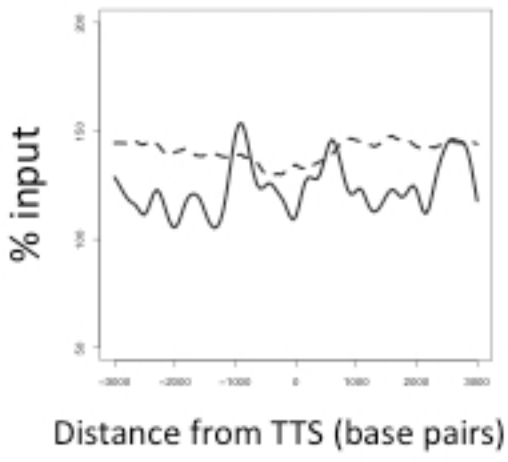

B

αH3K27me3

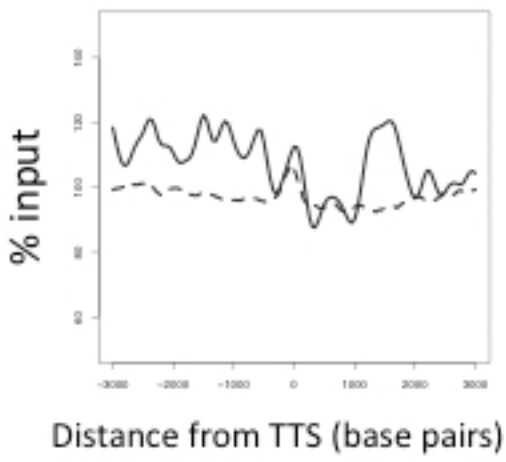
αH3K27ac

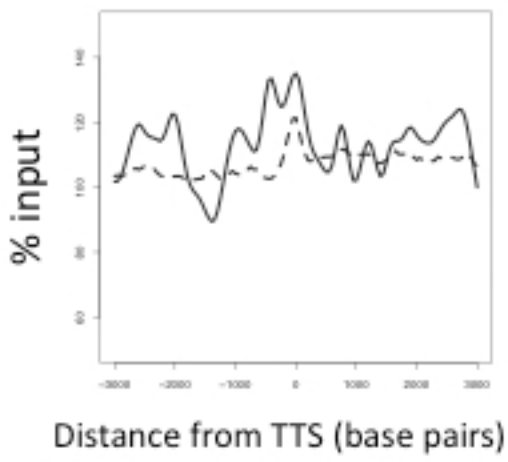
Wild Type

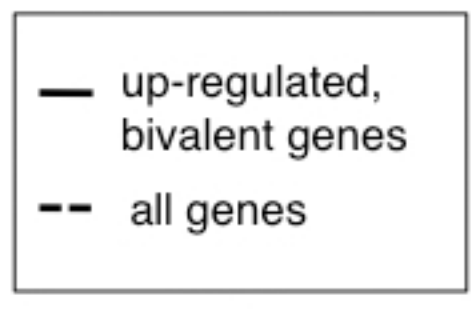


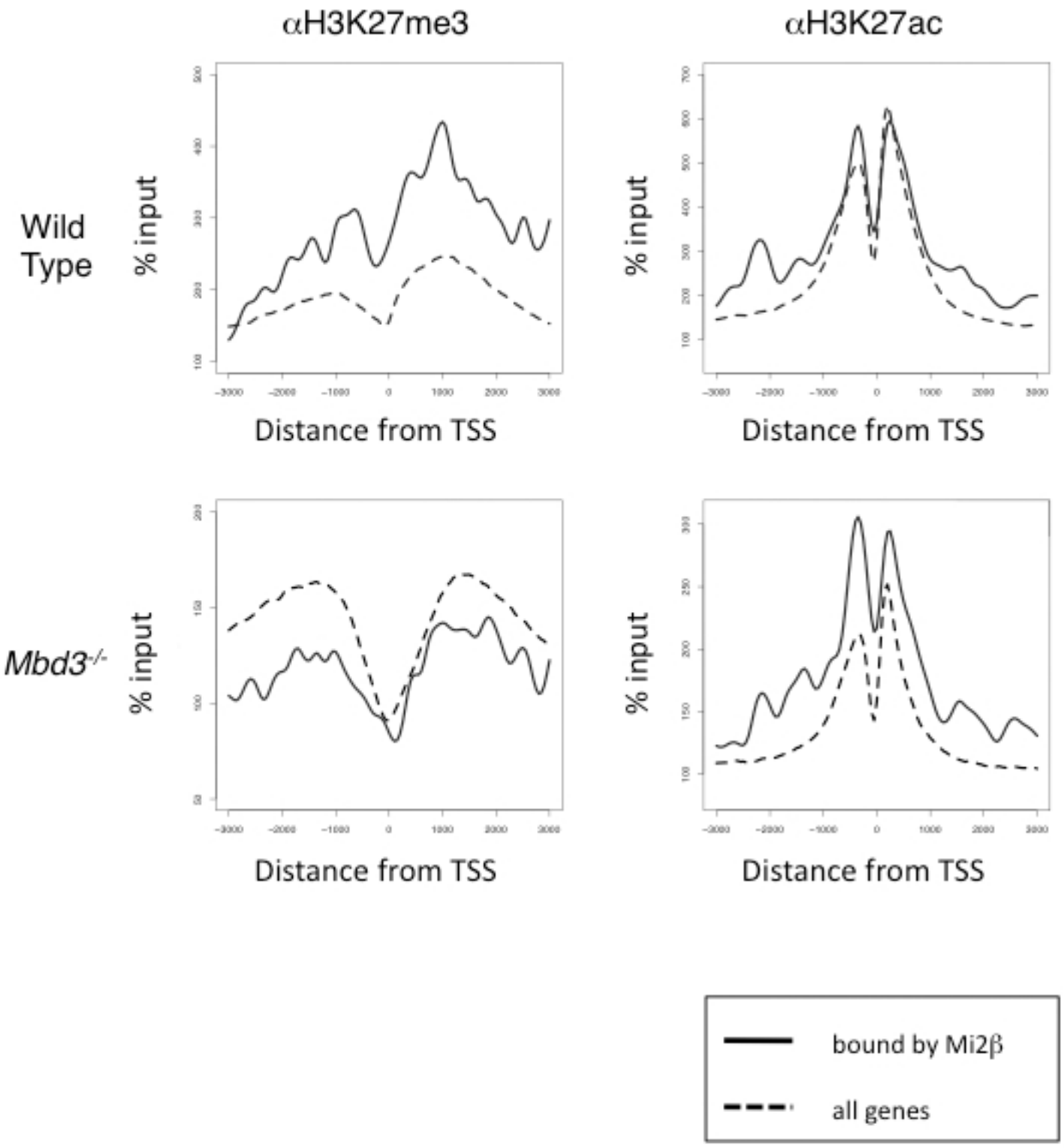




*Mbd3*$^{-/-}$

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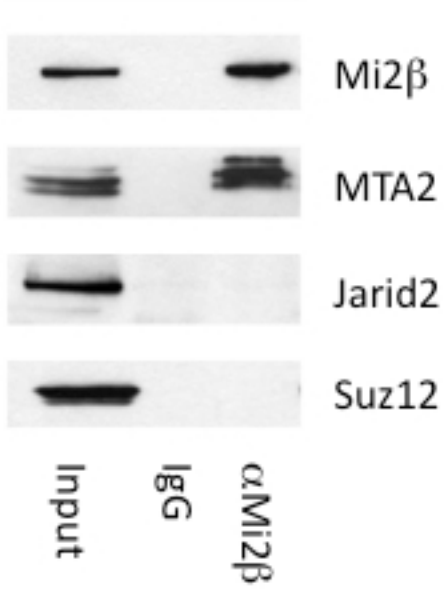
αH3K27ac
Wild Type
Mbd3-/-
% input
Distance from TSS
bound by Mi2β
all genes

**A**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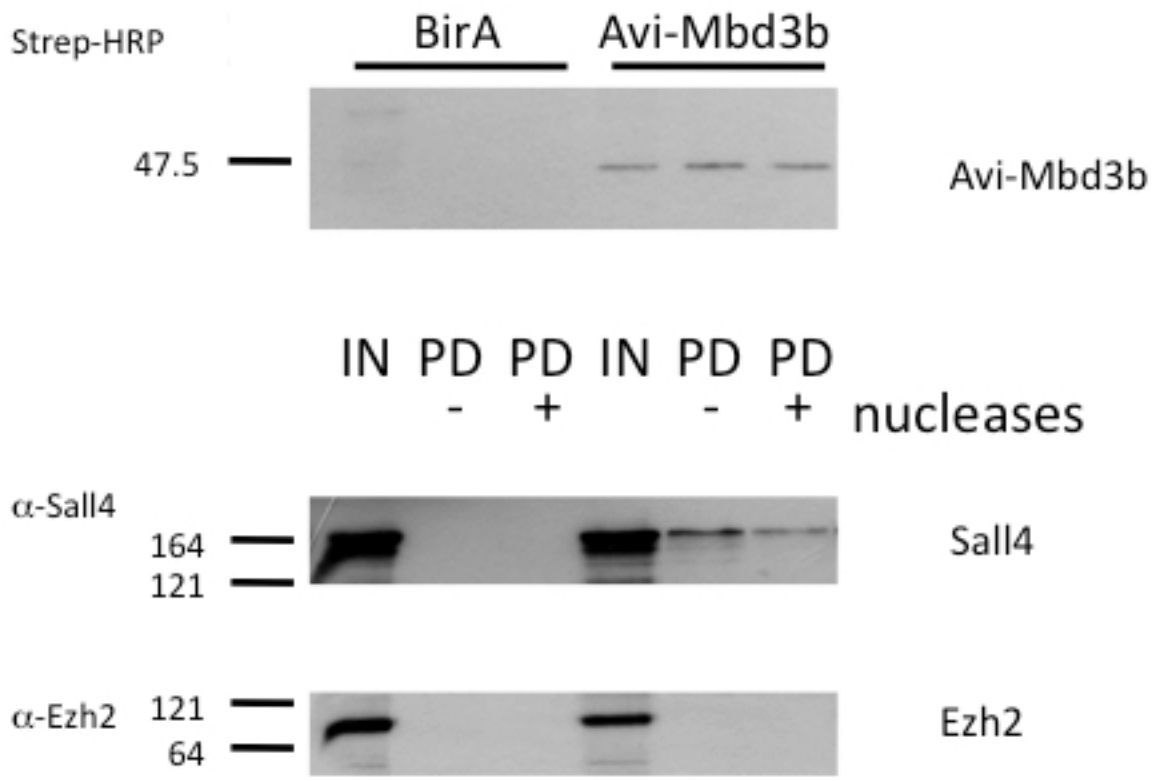
Jarid2
Suz12
Input
IgG
αMi2β

**B**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BirA
Avi-Mbd3b
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Avi-Mbd3b
IN PD PD IN PD PD
- + - + nucleases
α-Sall4
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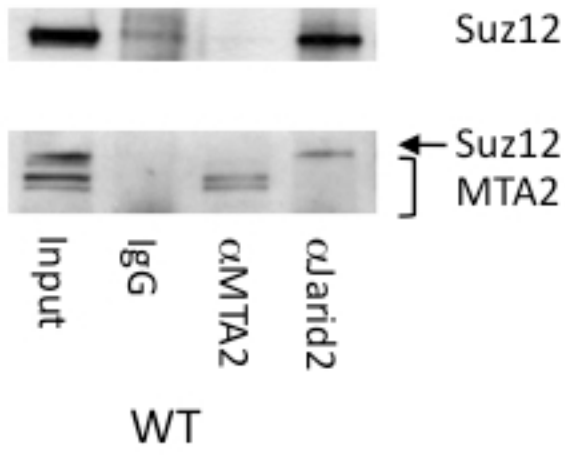
121
Sall4
α-Ezh2
121
64
Ezh2

**C**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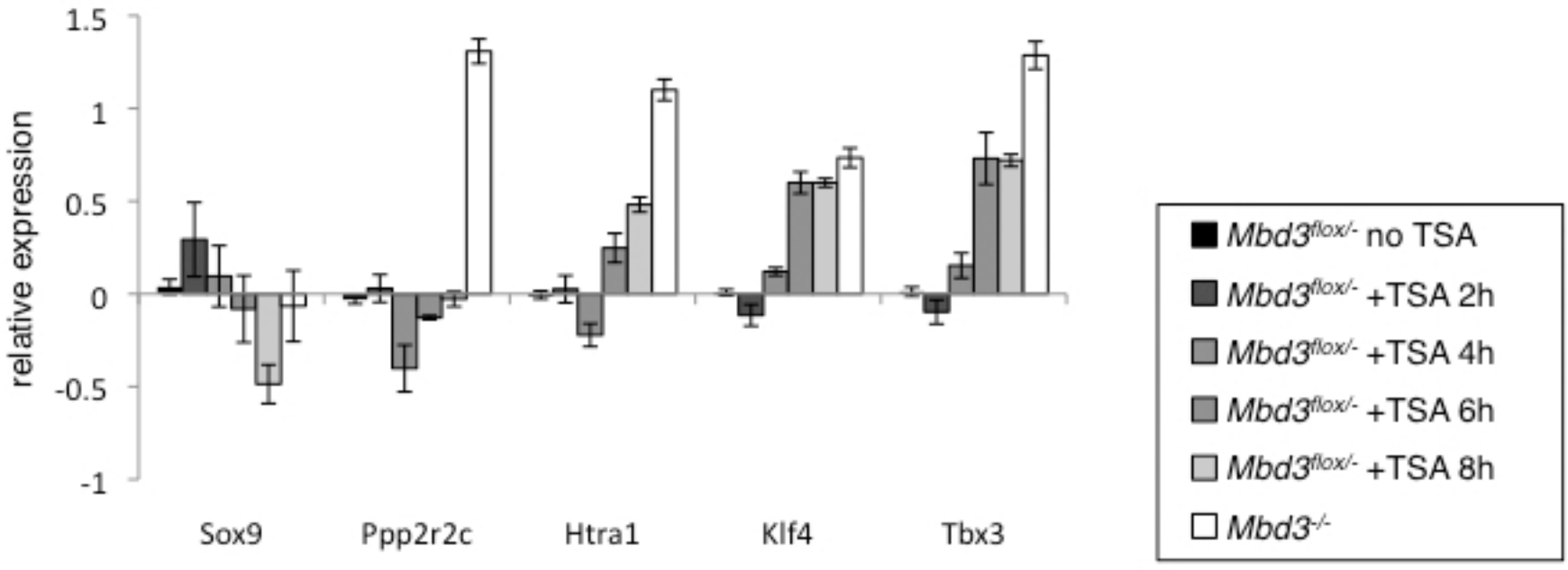
Input
IgG
αMTA2
αJarid2
WT

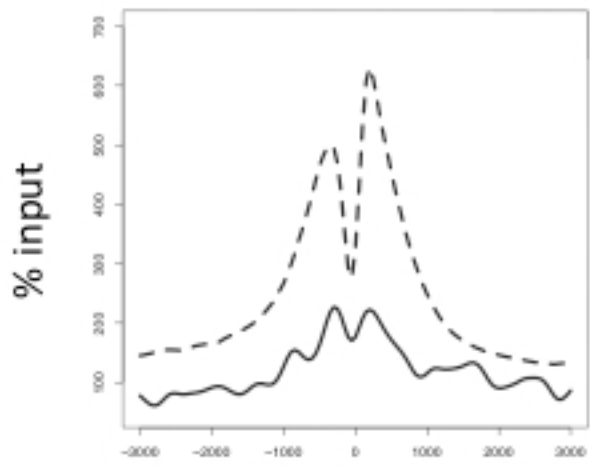
Reynolds_Supp Figure 5



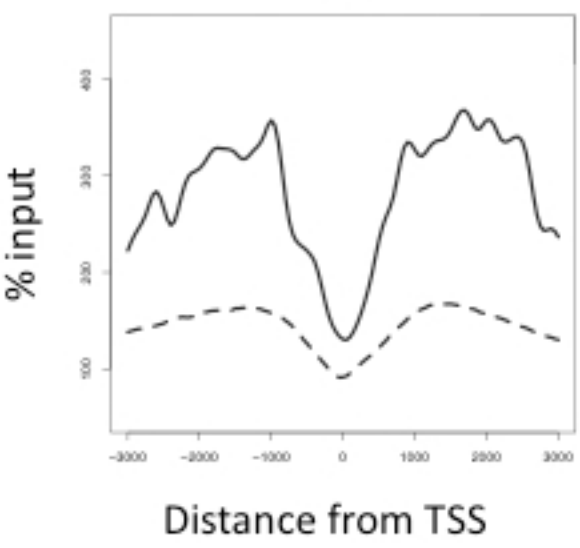
αH3K27me3

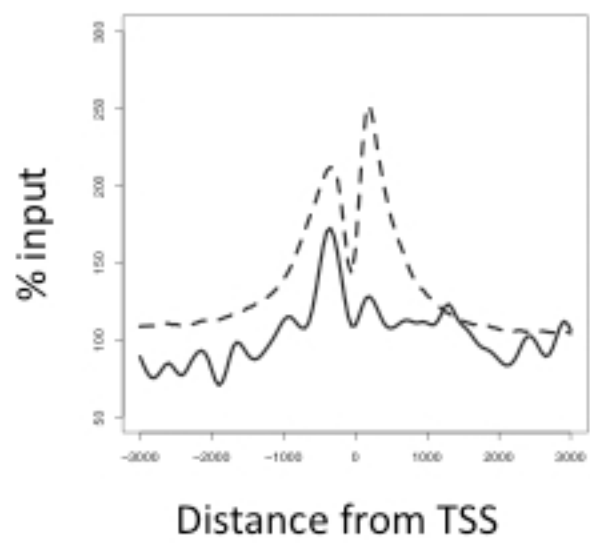
αH3K27ac

Wild Type


*Mbd3*$^{-/-}$




NuRD-dependent Suz12 target genes

all genes