

Supplemental Material

Histone Deacetylases maintain expression of the pluripotent gene network via recruitment of RNA polymerase II to coding and non-coding loci

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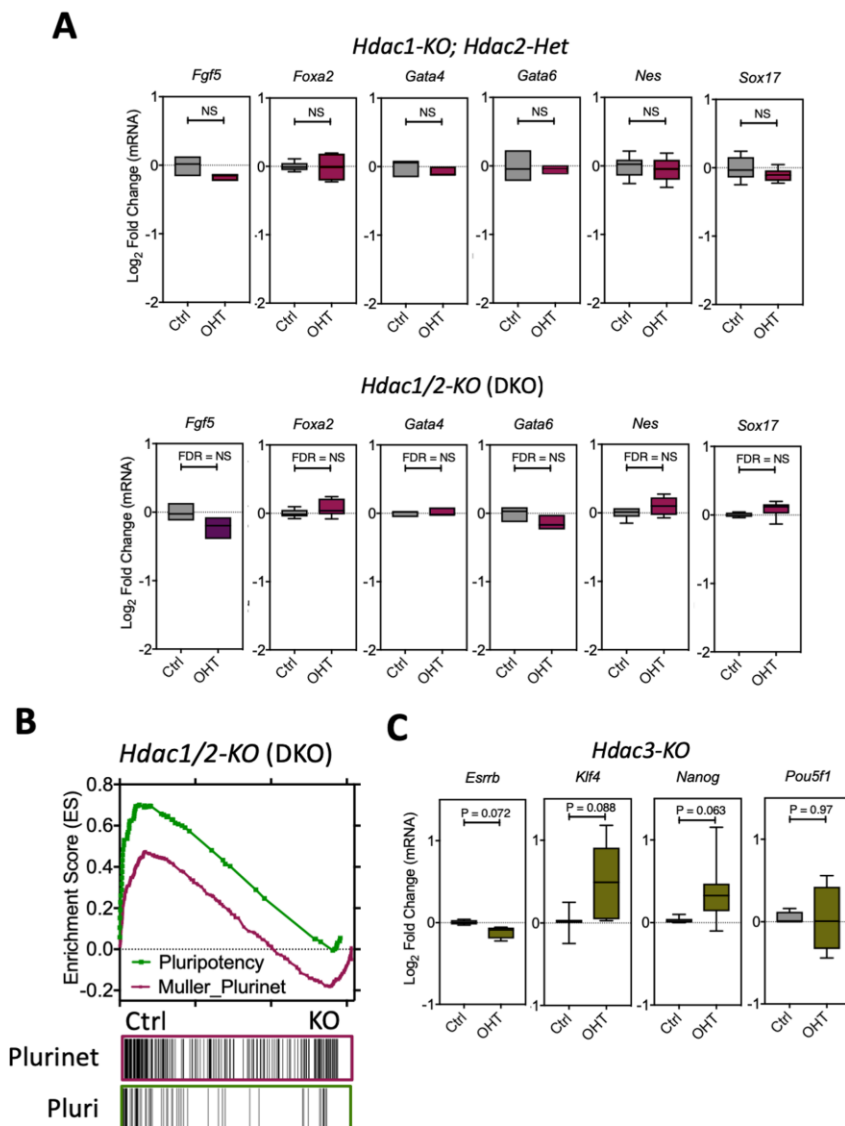
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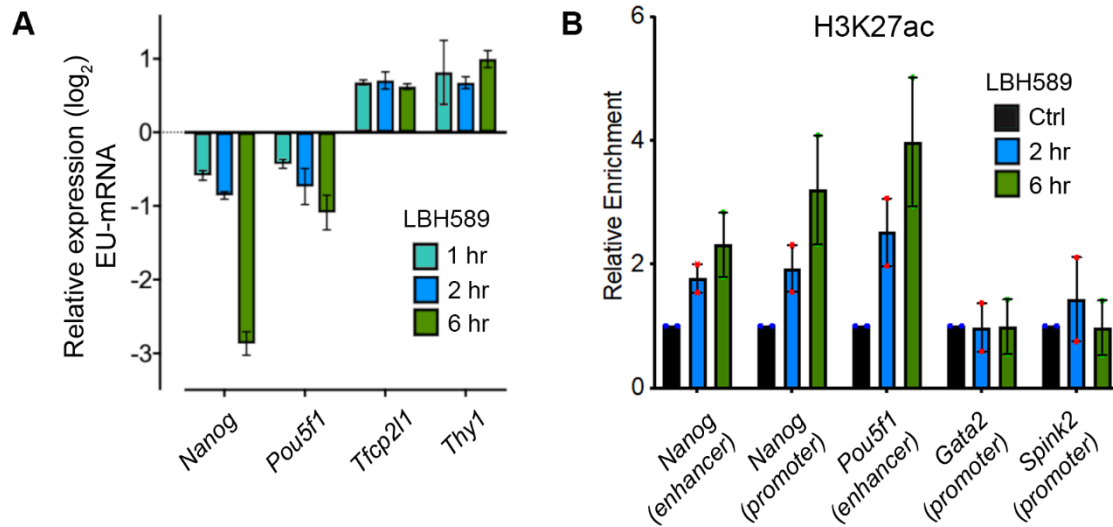
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Supplemental Figure S1



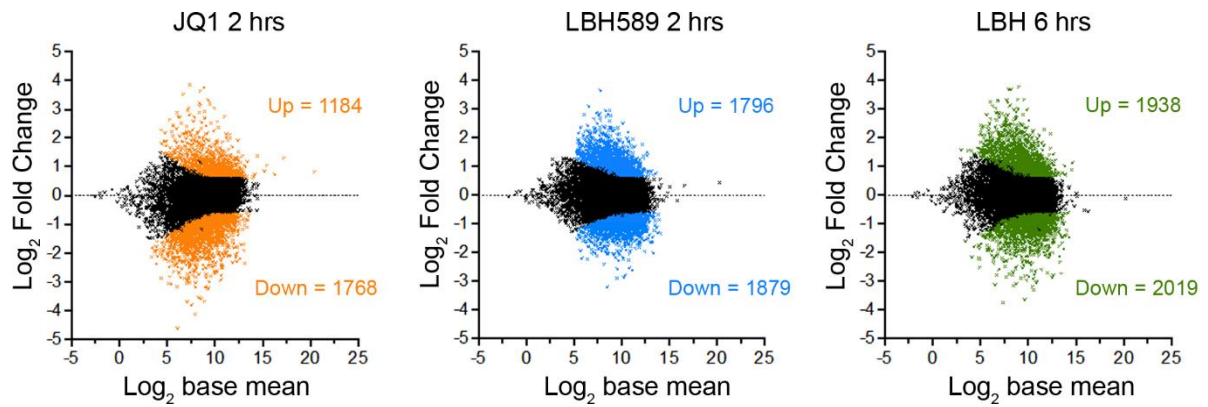
Supplemental Fig S1 – Loss of HDAC1/2 or HDAC3 does not lead to differentiation of embryonic stem cells (ESC). (A) Box plots generated from microarray data from compound (*Hdac1-KO; Hdac2-Het*) or double knockout (DKO) ESCs, treated with 4-hydroxytamoxifen (OHT) or and untreated control (Ctrl). Microarray data from $n = 3$ biological replicates were used to generate floating box plots (min-max) with line at mean. Statistical differences were calculated using Benjamini & Hochberg false discovery rate (FDR). (B) Gene set enrichment analysis (GSEA) plot of microarray data showing Ctrl samples are enriched for the Muller Plurinet ($ES=0.47$; $P<0.0001$) and pluripotency ($ES=0.70$; $P<0.0001$) gene sets compared to DKO cells. (C) Box plots generated from microarray data from *Hdac3-KO* ESCs, treated with 4-hydroxytamoxifen (OHT) or and untreated control (Ctrl). Microarray data from $n = 3$ biological replicates were used to generate floating box plots (min-max) with line at mean. Statistical differences were calculated using Benjamini & Hochberg false discovery rate (FDR).

Supplemental Figure S2



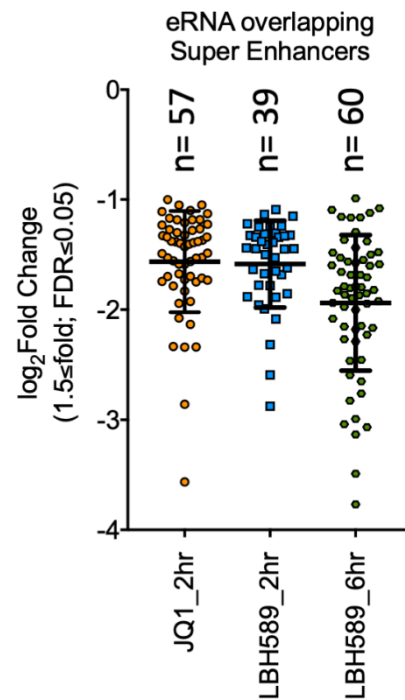
Supplemental Fig S2 – HDAC inhibition causes down-regulation of nascent pluripotent transcripts while increasing histone acetylation at promoters and enhancers. (A) RT-qPCR was used to quantify nascent RNA transcription for *Nanog*, *Pou5f1*, *Tfcp2l1* and *Thy1*. Values represent relative $\log_2$ mean ($\pm$ SEM) of $n = 3$ technical replicates. (B) ChIP-qPCR analysis of H3K27ac enrichment at the indicated regions. This graph shows $n=2$ biological replicates ($\pm$ SEM).

Supplemental Figure S3



Supplemental Fig S3 – HDAC inhibition causes approximately equal numbers of up- and down-regulated nascent protein-coding transcripts. MA plots for protein-coding transcripts using PRO-seq data from the indicated treatments.

Supplemental Figure S4



Supplemental Figure S4 - HDACs positively regulate a subset of eRNAs associated with super-enhancers. Scatter plots of average Log₂ fold-changes in eRNA transcripts which overlap super-enhancers following ESCs following treatment with JQ1 (2hr) or LBH589 (2hr and 6hr). Plots and the error bars represent mean $\pm$ SD. The number of SEs affected by each treatment is also shown.

Supplemental Table 1. A list of antibodies used in the study, with type, source and applications indicated.

Antibody	Source	Clonality	Company	Application	Concentration
HDAC1	Rabbit	Monoclonal	Abcam (ab109411)	Western Blotting	1:2500
HDAC2	Mouse	Monoclonal	Millipore (05-814)	Western Blotting	1:2000
NANOG	Rabbit	Polyclonal	Bethyl (A300-397A)	Western Blotting	1:2500
POU5F1 (OCT4)	Mouse	Monoclonal	Santa Cruz (SC5279)	Western Blotting	1:500
Tubulin alpha-4A	Mouse	Monoclonal	Millipore/Sigma (T5168)	Western Blotting	1:15000
RNA polymerase II	Mouse	Monoclonal	Active Motif (39097)	ChIP-qPCR	10 μ L /ChIP

Supplemental Table 2. A list of primers used in the study. Their sequence, annealing temperatures and applications are indicated.

Accession Number	Target	Primer Name	Sequence	Annealing temp	Application
NM_011934	<i>Esrrb</i>	Esrrb_R	CATGAAATGCCTCAA AGTGGG	58°C	RT-qPCR
		Esrrb_F	AAATCGGCAGGTTCA GGTAG		
NM_010637	<i>Klf4</i>	Klf4_F	TGTGTGCGAGGAAG AGGAAGC	59°C	RT-qPCR
		Klf4_R	ACGACTCACCAAGCA CCATCA		
NM_028016	<i>Nanog</i>	Nanog_F	AGGGTCTGCTACTGA GATGCTCTG	60°C	RT-qPCR
		Nanog_R	CAACCACTGGTTTTTC TGCCACCG		
NM_013633	<i>Pou5f1</i>	Pou5f1_F	AGTATGAGGCTACAG GGACA	60°C	RT-qPCR
		Pou5f1_R	CAAAGCTCCAGGTTC TCTTG		
NM_023755	<i>Tfcp2l1</i>	Tfcp2l1_F	AGGTGCTGACCTCCT GAAGA	58°C	RT-qPCR
		Tfcp2l1_R	GTTTTGCTCCAGCTCC TGAC		
NM_009382	<i>Thy1</i>	Thy1_R	TGCTCTCAGTCTTGCA GGTG	57°C	RT-qPCR
		Thy1_F	TGGATGGAGTTATCC TTGGTGTT		
NM_139218	<i>Dppa3</i>	Dppa3_F	TCGACCCAATGAAGG ACCCTGAAA	60°C	RT-qPCR
		Dppa3_R	TTGGGAAAGGCGCTT TGAACCTCC		

NM_008108	<i>Gdf3</i>	Gdf3_F	TAAGGATTGGAGCAG CAACCGACT	60°C	RT-qPCR
		Gdf3_R	ATGACGGTGGCAGA AGTTCCTACA		
NM_011443	<i>Sox2</i>	Sox2_F	TAGAGCTAGACTCCG GGCGATGA	60°C	RT-qPCR
		Sox2_R	TTGCCTTAAACAAGA CCACGAAA		
NM_007393	<i>Actb</i> (<i>β-ACTIN</i>)	b_Actin_F	GGCTCCTAGCACCAT GAAGA	60°C	RT-qPCR
		b_Actin_R	AGCTCAGTAACAGTC CGCCT		
NC_000072	<i>Nanog</i> <i>enhancer</i> (5kb)	Nanog_enhancer (5kb)_F	GTTTGGACTGCTAAC CACCCAGAG	58°C	ChIP_qPCR
		Nanog_enhancer (5kb)_R	GGCAGGCTTGCTACA TTCCTTATC		
NC_000072	<i>Nanog</i> <i>promoter</i>	Nanog_promoter _F	ACAGCTTCTTTTGCAT TACAATGTCC	58°C	ChIP_qPCR
		Nanog_promoter _R	TATTCTCCAGGCACC CAGGC		
NC_000083	<i>Pou5f1</i> <i>enhancer</i> (-2kb)	Pou5f1_enhancer (-2kb)_F	AGGTGCATGATAGCT CTGCC	58°C	ChIP_qPCR
		Pou5f1_enhancer (-2kb)_R	AAGCCGCCAAGTTCA CAAAG		
NC_000072	<i>Gata2</i> <i>promoter</i>	Gata2_promoter_ F	GGTCATTCCCGAAGT CCAGC	58°C	ChIP_qPCR
		Gata2_promoter_ R	GACTCCTGCACAGAC GTGAA		
NC_000071	<i>Spink2</i> <i>promoter</i>	Spink2_promoter _F	CCCCAAACACATCCTT TCGAC	58°C	ChIP_qPCR
		Spink2_promoter _R	TATGGAGTCTGTCCG GGGAA		
NC_000067	<i>Tfcp2l1</i> <i>enhancer</i> (-2kb)	Tfcp2l1_enhancer (-2kb)_F	AATCAAAAGGCTGCC CTTGC	58°C	ChIP_qPCR
		Tfcp2l1_enhancer (-2kb)_R	TCCCGGCCCTAAGAA CCTAA		
NC_000072	<i>Nanog</i> <i>TSS</i>	Nanog_TSS_F	GTGCAGCCGTGGTTA AAAG	58°C	ChIP_qPCR
		Nanog_TSS_R	GGTCCACCATGGACA TTGT		
NC_000070	<i>Klf4</i> TSS	Klf4_TSS_F	TAATTCTCGCTCGCT TGCT	58°C	ChIP_qPCR
		Klf4_TSS_R	CGTGCGCGGAGTTTG TTTAT		
NC_000083	<i>Pou5f1</i> <i>TSS</i>	Pou5f1_TSS_F	GATCCTCGAACCTGG CTAAG	58°C	ChIP_qPCR
		Pou5f1_TSS_R	CAGGCTGCAAAGTCT CCAC		