Supplemental Material

The Histone Variant macroH2A1 Regulates Target Gene Expression in Part by Recruiting the Transcriptional Coregulator PELP1

Hussey *et al.* (2014)

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1) Oligonucleotide Sequences

a) shRNAs:

Target	Sequence	Source
Luc	5'-gatatgggctgaatacaaa-3'	Reynolds *et al* (1)
PELP1 #1	5'-ggagcattcagcaggtgttac-3'	Invitrogen BLOCK-iT RNAi Designer*
PELP1 #2	5'-ggaccaaggtgtatgcgatat-3'	Dimple *et al* (2)
mH2A1 #1	5'-gcaatgcagcgagagacaaca-3'	Gamble *et al* (3)
mH2A1 #2	5'-gcgtgtgttgtggtgctttat-3'	Gamble *et al* (3)

*Sequences were chosen based on priority score according to criteria described at the website (https://rnaidesigner.invitrogen.com/rnaiexpress).

b) RT-qPCR:

Gene Name	**Primer Sequence**
β-ACTIN forward	5'-AGCTACGAGCTGCCTGAC-3'
β-ACTIN reverse	5'-AAGGTAGTTTCGTGGATGC-3'
AREG forward	5'-GAGAAGCTGAGGAACGAAAG-3'
AREG reverse	5'-GGCTATGACTTGGCAGTGAC-3'
ASCL1 forward	5'-ACTGGGACCTGAGTCAATGC-3'
ASCL1 reverse	5'-GCTGTGCGTGTTAGAGGTGA-3'
CCL2 forward	5'-CCCCAGTCACCTGCTGTTAT-3'
CCL2 reverse	5'-GCTTCTTTGGGACACTTGCT-3'
H2AFY forward	5'-AAGAAGGGACGGGTCACAC-3'
H2AFY reverse	5'-GGGTGGATGTTGGGTAACAC-3'
IL24 forward	5'-AGGCGGTTTCTGCTATTC-3'
IL24 reverse	5'-CTGCATCCAGGTCAGAAG-3'
MAPK12 forward	5'-ACCTGGCTGTGAACGAAG-3'
MAPK12 reverse	5'-CACCACGTACCCAGTCATC-3'
NELL2 forward	5'-TGAAGGGAACCACCTACC-3'
NELL2 reverse	5'-ATTTGCCATCCACATACG-3'
PARP1 forward	5'-GTGTGGGAAGACCAAAGGAA-3'
PARP1 reverse	5'-TTCAAGAGCTCCCATGTTCA-3'
PELP1 forward	5'- CACCAGAGACACCTGCAGAA-3'
PELP1 reverse	5'- AGCTGTGTCATCCTGCTCCT-3'
SOCS2 forward	5'-ACACGTCAGCACCATCTCTG-3'
SOCS2 reverse	5'-TGGCACCGGTACATTTGTTA-3'
TFF1 forward	5'-TGCTTCTATCCTAATACCATCG-3'
TFF1 reverse	5'-AGATCCCTGCAGAAGTGTC-3'
TMOD3 forward	5'-GGAAGTAGTAATGGTGTTGACC-3'
TMOD3 reverse	5'-GCTCATCAAATACCGGAAG-3'

TNFSF10 forward	5’-TCACATAACTGGGACCAGAG-3’
TNFSF10 reverse	5’-AGTTCACCATTCCTCAAGTG-3’
TSPAN5 forward	5’-ACACTGGACAGACCCAGCTT-3’
TSPAN5 reverse	5’-TGTCAGTGAGCAGCATTTCC-3’

c) ChIP-qPCR:

Gene Name	**Primer Sequence**
AREG -3.0 kb forward	5’-TTGTTCCCCTTTGTCTCTGC-3’
AREG -3.0 kb reverse	5’-GATGTGTCATGGCATTCTGG-3’
AREG +1.5 kb forward	5’-TTCCCCTGTGAGTGAAATGC-3’
AREG +1.5 kb reverse	5’-AGCCAGGTATTTGTGGTTCG-3’
ASCL1 -2.0 kb forward	5’-GACTCTGCTTTTGGGTGCTC-3’
ASCL1 -2.0 kb reverse	5’-TTCACACCTCAGGCCTTTCT-3’
ASCL1 +2.0 kb forward	5’-GAGCAACTGGGACCTGAGTC-3’
ASCL1 +2.0 kb reverse	5’-CTATAACGCGTGTGCTGCTC-3’
CCL2 -1.5 kb forward	5’-ACCTAACGAAAGCTGGGTTG-3’
CCL2 -1.5 kb reverse	5’-TTCAGGGAGTCAGGTATGGTG-3’
CCL2 +1.0 kb forward	5’-TGGCCTGAAGTTCTTCCTTG-3’
CCL2 +1.0 kb reverse	5’-GGGGTTCACTTTTTCCCTTG-3’
IL24 +2.0 kb forward	5’-AGGGCCAAGAATTCCACTTT-3’
IL24 +2.0 kb reverse	5’-GTCTTTCACAGCCCAGAAGG-3’
MAPK12 +3.0 kb forward	5’-TTCACCCCAACCAAACAGA-3’
MAPK12 +3.0 kb reverse	5’-CTCTTTCCATAGCGCTGTCC-3’
NELL2 -1.5 kb forward	5’-TCAGAATTCGGGAGCTCTTT-3’
NELL2 -1.5 kb reverse	5’-TTTCAAATTTGGGAAATTGCAT-3’
SOCS2 -2.0 kb forward	5’-AAGTTCTCTGGAAGCCACAGG-3’
SOCS2 -2.0 kb reverse	5’-CGGTGAGTTTGGATTTTTCTG-3’
SOCS2 +4.0 kb forward	5’-AGTTCTCTCGCTTGCGATTC-3’
SOCS2 +4.0 kb reverse	5’-ACCGGGATACTTGCAATCTG-3’
TFF1 -3.0 kb forward	5’-TGGGTTCCGCCCACTCT-3’
TFF1 -3.0 kb reverse	5’-CTGCCCCCGGGACTCT-3’
TMOD3 -2.5 kb forward	5’-TCTGGCCCCATATGTGGTAT-3’
TMOD3 -2.5 kb reverse	5’-TGCAGTTTGATGGTGGATTT-3’
TNFSF10 -2.0 kb forward	5’-AGGCATGAAACGAAGGAATG-3’
TNFSF10 -2.0 kb reverse	5’-CTTGACCTGACCCCGAGATA-3’
TSPAN5 -3.0 kb forward	5’-TTGACTCAGCAGTCCCTCTTC-3’
TSPAN5 -3.0 kb reverse	5’-TCCATCTGCTGGGGTATTTC-3’
NFIB -3.0 kb forward	5’-CTGGTTGCACTTTGATGACC-3'
NFIB -3.0 kb reverse	5’-TTTTCCTCGCCTCACCATAC-3’
COLEC10 -3.0 kb forward	5’-GCAAAAACAGTGTTGGCTTG-3’
COLEC10 -3.0 kb reverse	5’-AAAAATAGTGCTTCCCAGAAAGG-3’
COL14A1 -1.0 kb forward	5’-TGGAATGTCGGAGGGACTAC-3’
COL14A1 -1.0 kb reverse	5’-TTTCCAAATTTGCCTCCATC-3’
KCNK2 promoter forward	5’-ATGGGACGATGGCTTGTTAG-3’

KCNK2 promoter reverse	5’-GAAATCCCTTTTTGCTCGTG-3’
RNF128 promoter forward	5’-GACGTGAGTGGACAATGGTG-3’
RNF128 promoter reverse	5’-CCTGGTTCATTGCTCATTCAT-3’
HMCN1 promoter forward	5’-GGCAGATAGCAGTCCAGGAG-3’
HMCN1 promoter reverse	5’-AAAAAGTTTGGCGCTGAGAA-3’
IL8 +1.0 kb forward	5’-GTGCAGTTTTGCCAAGGAGT-3’
IL8 +1.0 kb reverse	5’-GGGTGGAAAGGTTTGGAGTA-3’

2) Supplemental References

1. **Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A.** 2004. Rational siRNA design for RNA interference. Nat Biotechnol **22:**326-330.
2. **Dimple C, Nair SS, Rajhans R, Pitcheswara PR, Liu J, Balasenthil S, Le XF, Burow ME, Auersperg N, Tekmal RR, Broaddus RR, Vadlamudi RK.** 2008. Role of PELP1/MNAR signaling in ovarian tumorigenesis. Cancer Res **68:**4902-4909.
3. **Gamble MJ, Frizzell KM, Yang C, Krishnakumar R, Kraus WL.** 2010. The histone variant macroH2A1 marks repressed autosomal chromatin, but protects a subset of its target genes from silencing. Genes Dev **24:**21-32.

3) Supplemental Figure Legends
(the figures are shown in order beginning on page 8)

Fig. S1. Comparison of two independent shRNA target sequences for the inducible knockdown of PELP1.
A) Knockdown of PELP1 by two independent shRNA target sequences. Whole cell lysates or acid extracted pellets (for mH2A1, *e.g.*) were collected from doxycycline-treated Luc_i or $PELP1_i$ stable shRNA-mediated knockdown cell lines. Two independent shRNA sequences (#1 and #2) targeting PELP1 were analyzed by Western blotting for their ability to knockdown PELP1 relative to the Luc control. PARP-1 and β-actin were also analyzed as loading controls.
B) RT-qPCR analysis confirms the knockdown of PELP1 mRNA in the knockdown cell lines described in (A). Total RNA was isolated from Luc_i and $PELP1_i$ knockdown cells, reverse transcribed, and subjected to RT-qPCR using gene-specific primers to PELP1. Each bar is the mean + SEM for three independent RNA isolations.
C) Comparable effects on gene expression for each $PELP1_i$ shRNA sequence. Total RNA was isolated from Luc_i and $PELP1_i$ knockdown cells, reverse transcribed, and subjected to RT-qPCR using gene-specific primers. The effect of each knockdown (PELP1 shRNA #1 or PELP1 shRNA#2) was compared for a subset of target genes identified in Fig. 5. Each bar represents the mean + SEM from three or more independent determinations.
D) A correlation analysis comparing the effects of PELP1 shRNA #1 and PELP1 shRNA #2 at 22 genes indicates that both shRNAs produce similar effects on gene expression. The Spearman correlation coefficient (c.c.) and p-value are indicated. For all experiments described in the main text, only shRNA #2 was used. Luc, luciferase.

Fig. S2. No-antibody ChIP controls for PELP1 and macroH2A1.
Boxplots summarizing ChIP data from (A) 8 loci in MCF-7 cells and (B) 7 loci in A549 cells for no-antibody controls (NA), macroH2A1 (mH2A1) and PELP1. In both cell lines, the signal from the no-antibody controls are a fraction of that for the macroH2A1 and PELP1 ChIPs.

Fig. S3. The macrodomain of the histone variant macroH2A1.1 interacts with a both chromatin-associated proteins and nucleolar components.
A) Silver stain image of GST-macro1.1 interacting proteins from a GST pull-down assay. GST alone or GST-macro1.1 fusion protein were immobilized on glutathione affinity resin and incubated with or without HeLa nuclear extract. Bound fractions were washed, eluted with free glutathione, and separated by SDS-PAGE. Resulting eluates were visualized by silver stain. GST-macro1.1-specific protein bands were excised from the gel, trypsinized, and identified by mass spectrometry. All identified macrodomain-interacting proteins are described further in Supplemental Table S1.
B) Western blotting confirms the interaction of GST-macro1.1 with nucleolin and SET.

Fig. S4. MacroH2A domain and variant specificity for PELP1 interactions.
A) Schematic diagram of the histone variant macroH2A and GST-tagged macroH2A fusion constructs. The histone H2A-like region (H2A), macrodomain, and the basic linker region (Basic), are shown. The GST-macro1.1 fusion consists of GST fused to the non-histone regions (linker and macrodomain; amino acids 123 - 368). The GST-macro1.1 –linker has GST fused to the macrodomain of macroH2A1.1 only (amino acids 161 - 368). The GST-macro1.2 construct

has GST fused to the linker and macrodomain of macroH2A1.2 (amino acids 123 - 371). The GST-macro2 construct has GST fused to the liner and macrodomain of macroH2A2 (amino acids 123 - 372. GST alone, used as a control, is shown for comparison

B) Western blot for PELP1 from GST pull-down assays using the GST fusion constructs described in panel A. Coomassie stain demonstrates the relative loading of the GST fusion proteins. The data in this panel demonstrates that PELP1 binds specifically to the macrodomain of macroH2As and not to the basic linker region. In addition, the pull-down assay demonstrate that PELP1 can bind to all three macroH2A variants with differing affinity.

C) Western blots for rPARP-1 from GST pull-down assay as described in Fig. 1C. 20 μM ADPR or NAD+ was added as indicated. Bacterially purified recombinant PARP-1 (rPARP-1) was added to all pulldowns. The data demonstrates that PARP-1 can directly interact with the macrodomain of macroH2A1.1.

Fig. S5. PELP1 binding across 1% of the human genome represented by the 44 ENCODE regions.

Fourty-four histograms depicting the Log_2 ratios of PELP1 and macroH2A1 ChIP-chip signals from MCF-7 cells across all ENCODE regions. The genomic location and position and orientation of RefSeq genes are depicted below each track. The genes are color-coded according to expression microarray data: green, expressed; blue, unexpressed; grey, ambiguous. mH2A, macroH2A1; PELP, PELP1.

Fig. S6. PELP1 binding across several gene-specific loci.

Eleven histograms depicting the Log_2 ratios of PELP1 and macroH2A1 ChIP-chip signals from MCF-7 cells across the genes focused on within this manuscript. The genomic location and position and orientation of RefSeq genes are depicted below each track. They are color-coded according to expression microarray data: green, expressed; blue, unexpressed; grey, ambiguous. mH2A, macroH2A1; PELP, PELP1.

Fig. S7. Reductions in both mH2A1 and PELP1 upon mH2A1 depletion are not due to loss of nucleosomes.

The occupancy of histone H3 was examined by ChIP analyses in the Luc (*black bars*) and mH2A1 (*white bars*) knockdown cell lines. Each bar represents the mean + SEM (*error bars*) from two independent determinations. All changes in occupancy for H3 are not statistically different then the control cell line, as determined by a Student's *t*-test with a p-value threshold of < 0.05. Luc, luciferase; mH2A1, macroH2A1; KD, knockdown.

Fig. S8. PELP1 is recruited to gene promoter by macroH2A1 in A549 lung cancer cells.

A549 lung cancer cells were cultured in RPMI supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% FBS. The Luc and macroH2A1 knockdown A549 cell lines were constructed similarly to the MCF-7-derived lines described above.

A) Western blot showing the shRNA-mediated depletion of macroH2A1 in A549 cells compared to luciferase (Luc) knockdown cells. Histone H3 was analyzed as a loading control.

B and C) The occupancy of macroH2A1 (B) and PELP1 (C) was examined by ChIP analyses in the Luc (black bars) and macroH2A1 (white bars) knockdown cell lines. Each bar represents the mean + SEM (error bars) from three or more independent determinations. All changes in occupancy for macroH2A1 (B) and PELP1 (C) upon macroH2A1 knockdown are statistically

different then the control cell line, as determined by a Student's t-test with a p-value threshold of < 0.05. Luc, luciferase; mH2A1, macroH2A1; KD, knockdown.

Fig. S9. PELP1 acts to potentiate the transcriptional induction of the serum starvation-responsive gene, *SOCS2*.
A) macroH2A1 and PELP1 knockdown represses the induction of the serum starvation-responsive gene, *SOCS2*. Luc and macroH2A1 knockdown cells and Luc_i and $PELP1_i$ knockdown cells (+ dox) were cultured with serum (+) or serum deprivation (–) for 24 hrs. Total RNA was isolated, reverse-transcribed, and subjected to qPCR using gene-specific primers. *ASCL1* is shown as a control, as it is only modulated by macroH2A1. Each bar represents the mean + SEM (*error bars*) from three or more independent determinations. Bars marked with an *asterisk* are statistically different from the corresponding luciferase knockdown control (Luc, in the case of macroH2A1; Luc_i in the case of $PELP1_i$), as determined by a Student's *t*-test with at p-value threshold of < 0.05. The Luc and macroH2A1 knockdown data for *SOCS2* and *ASCL1* is taken from Gamble *et al.* (3).
B) MacroH2A1 and PELP1 occupancy are unaltered at the promoters of serum starvation-responsive genes. MacroH2A1 and PELP1 ChIP assays were performed from parental MCF-7 cells were cultured with serum (+) or serum deprivation (–) for 24 hrs. Primers were designed to regions upstream and downstream of the TSS. Each bar represents the mean + SEM (*error bars*) from three or more independent determinations. The macroH2A1 ChIP data for *SOCS2* and *ASCL1* is taken from Gamble *et al.* (3). Luc, luciferase; mH2A1, macroH2A1; i, inducible; U, upstream of the TSS; D, downstream of the TSS.

Fig. S10. TPA and serum starvation does not cause loss of nucleosomes at target gene promoters.
H3 ChIP was performed using MCF-7 cells. Primers were designed to regions upstream and downstream of the TSS. Each bar represents the mean + SEM (*error bars*) from three or more independent determinations. U, upstream of the TSS; D, downstream of the TSS.
A) MCF-7 cells treated with vehicle (–) or 100 nM TPA (+) for 1.5 hrs.
B) MCF-7 cells treated with with (+) or without (–) serum for 24 hrs.

4) Supplemental Figures 1 through 10
(presented in order on the following pages)

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Fig. S6. PELP1 binding across several gene-specific loci

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Fig. S8. PELP1 is recruited to gene promoters by macroH2A1 in A549 lung cancer cells

Fig. S9. PELP1 acts to potentiate the transcriptional induction of the serum starvation-responsive gene, *SOCS2*

Fig. S10. TPA and serum starvation does not cause loss of nucleosomes at target gene promoters

5) Supplemental Tables
(presented in order in a separate Excel file)

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Table S3. Gene lists used for gene ontology analyses in Table 1

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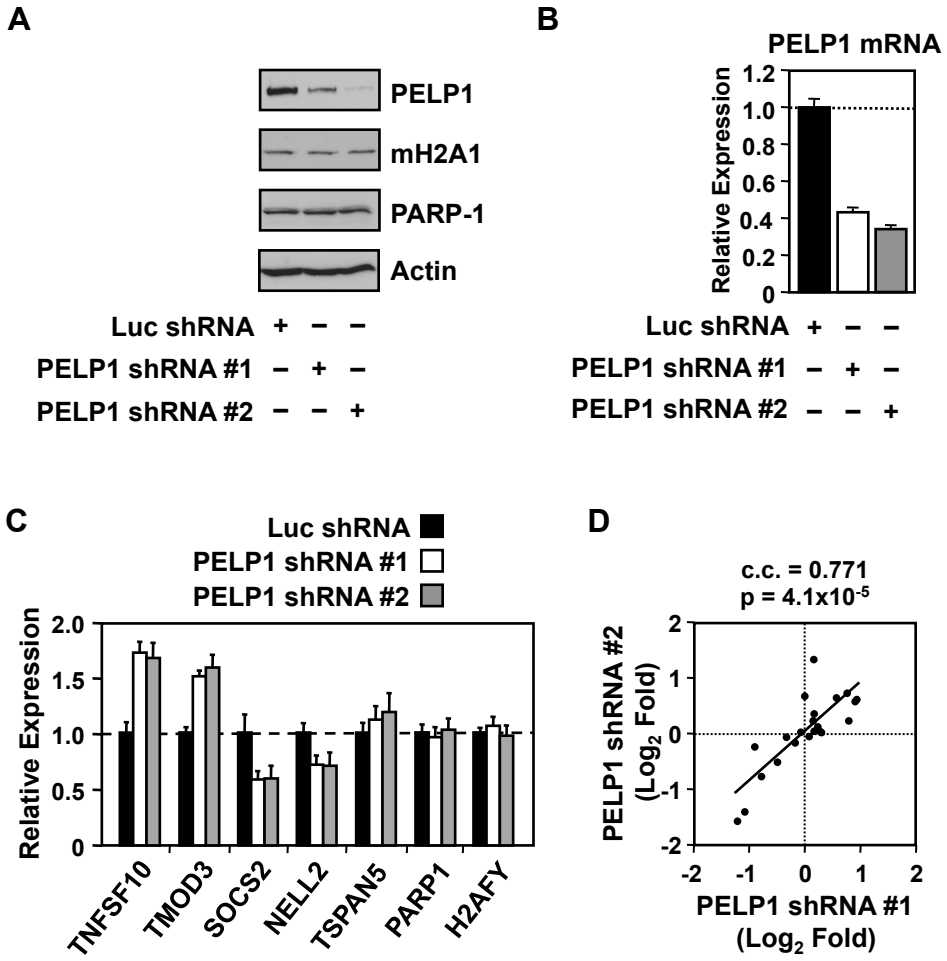


Fig. S1 – Hussey *et al.* (2014)

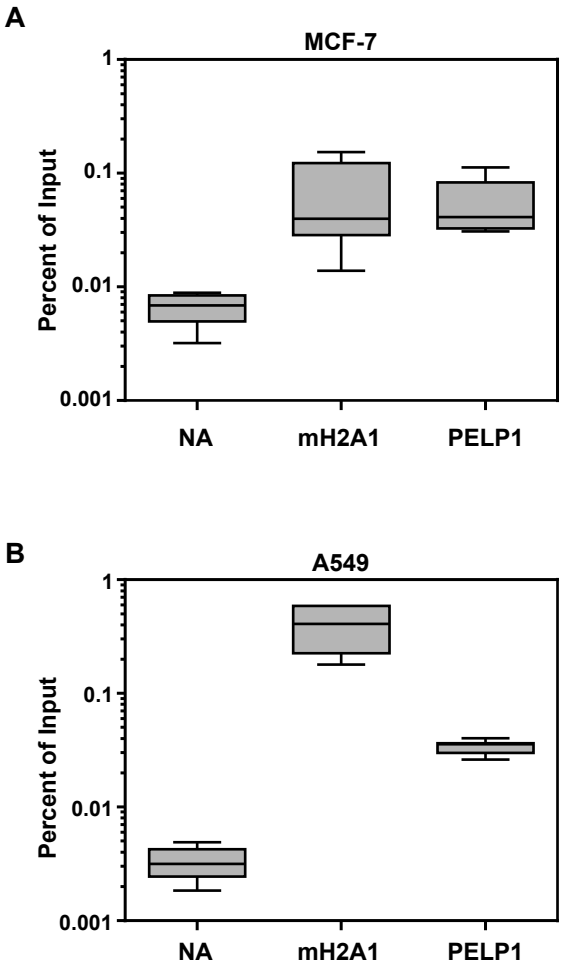


Fig. S2 – Hussey *et al.* (2014)

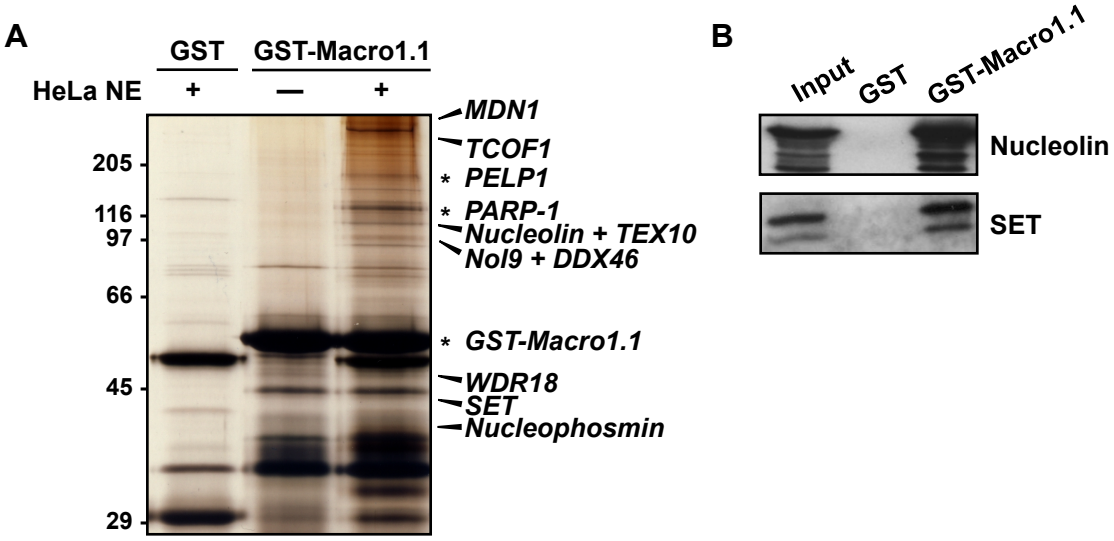


Fig. S3 – Hussey *et al.* (2014)

Fig. S4 – Hussey *et al.* (2014)

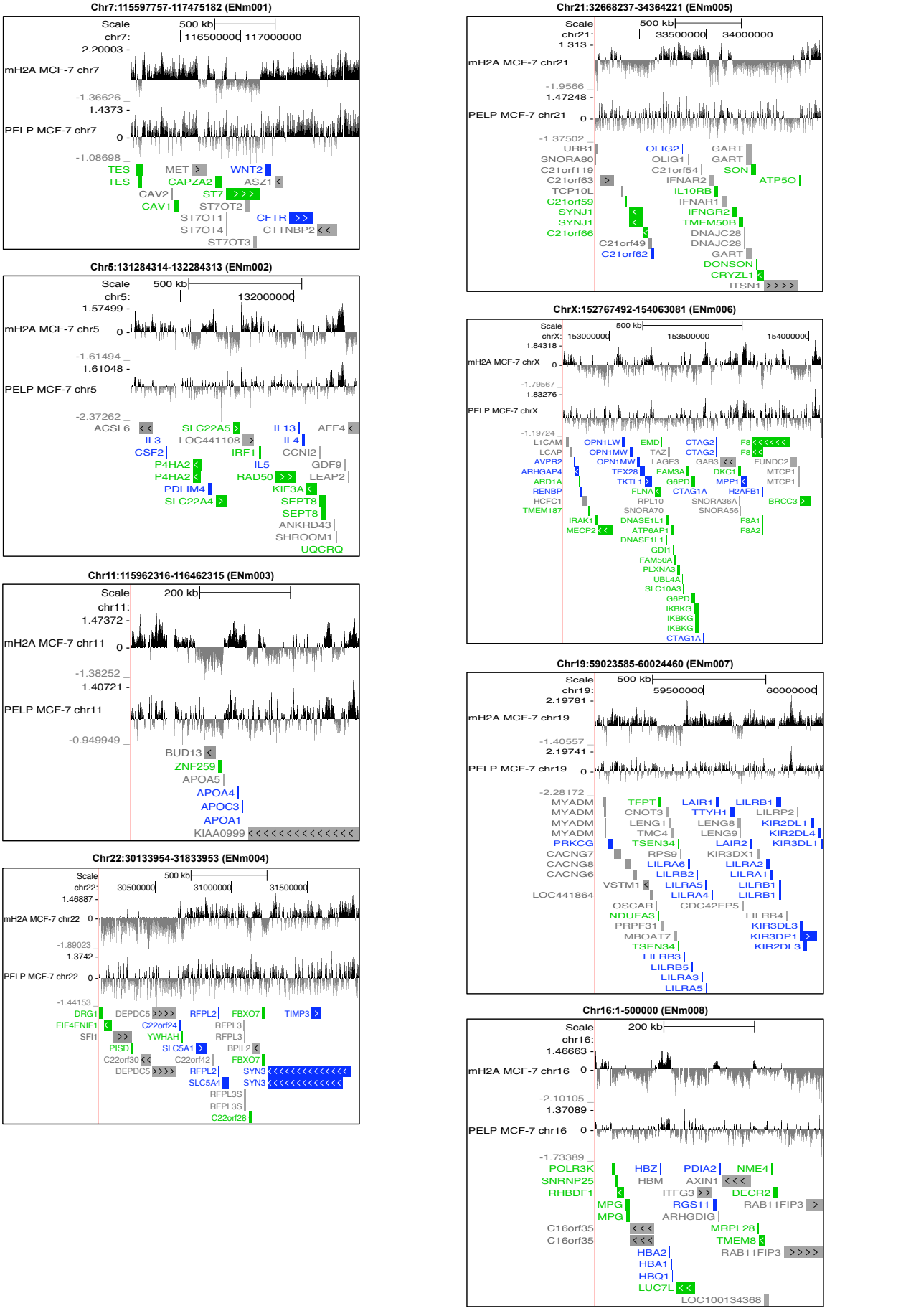


Fig. S5 – Hussey *et al.* (2014)

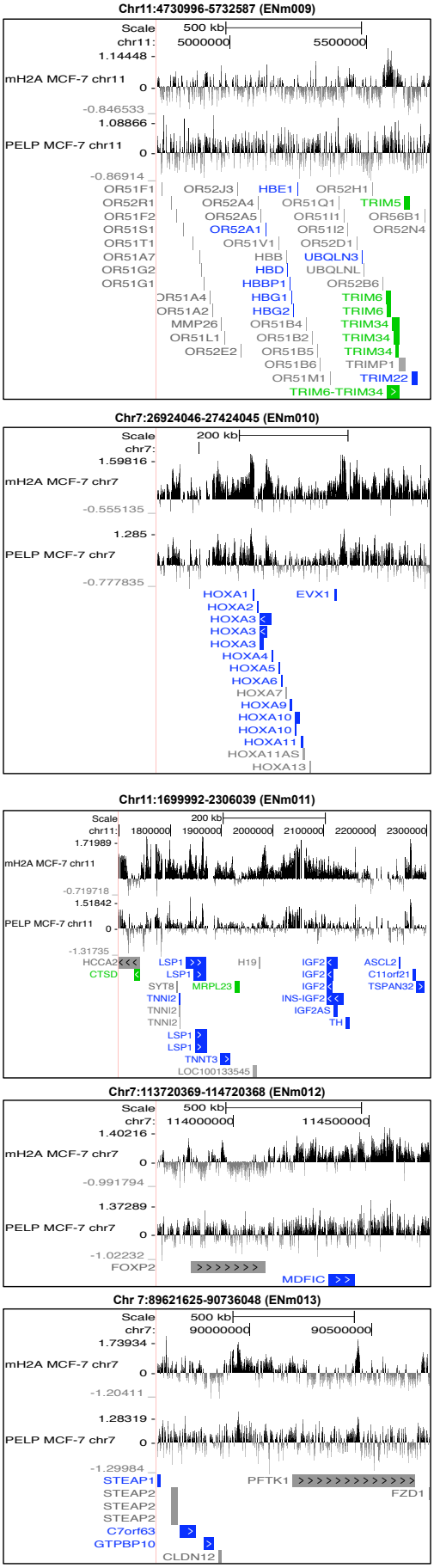
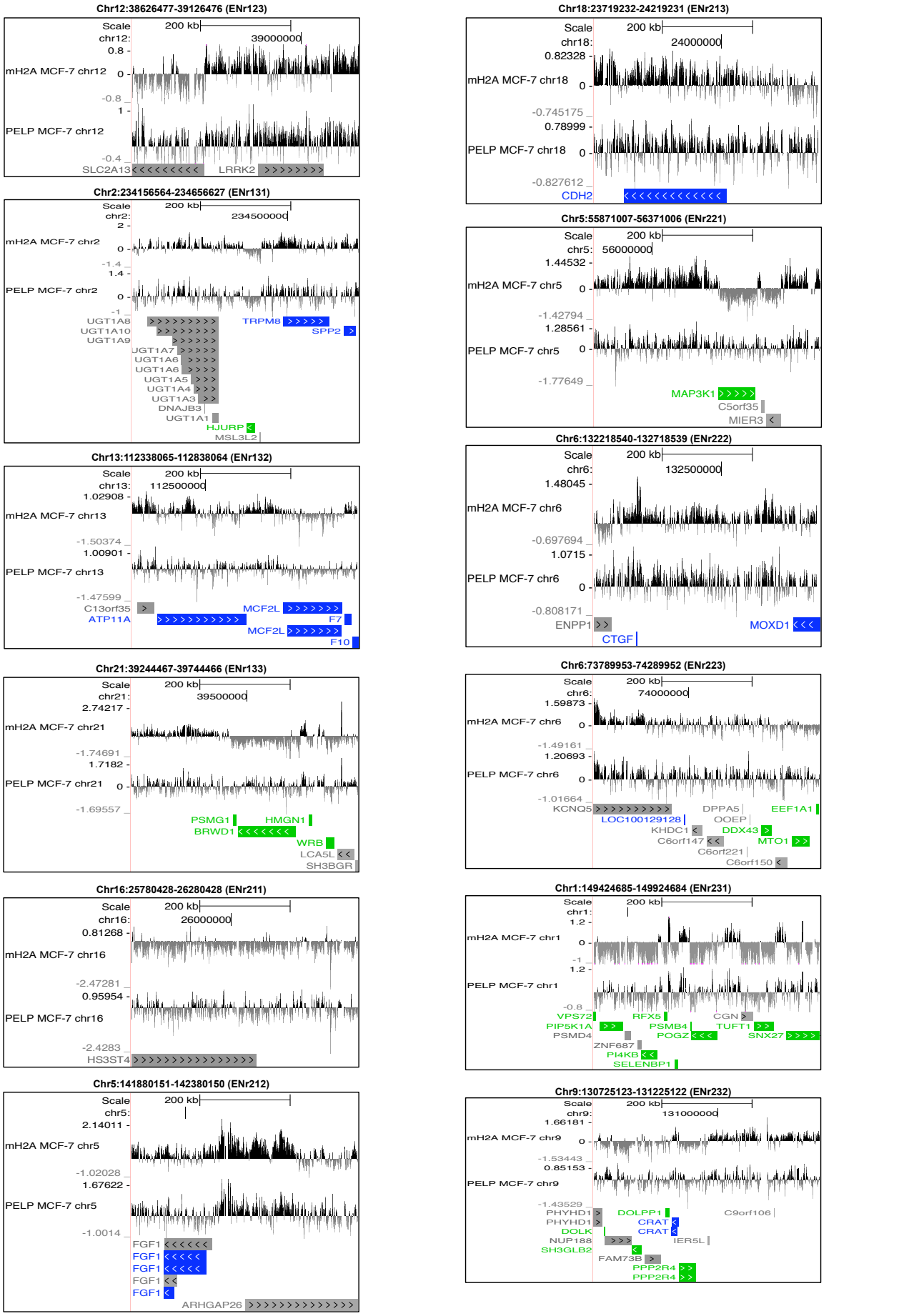


Fig. S5 – Hussey *et al.* (2014) - *Continued*



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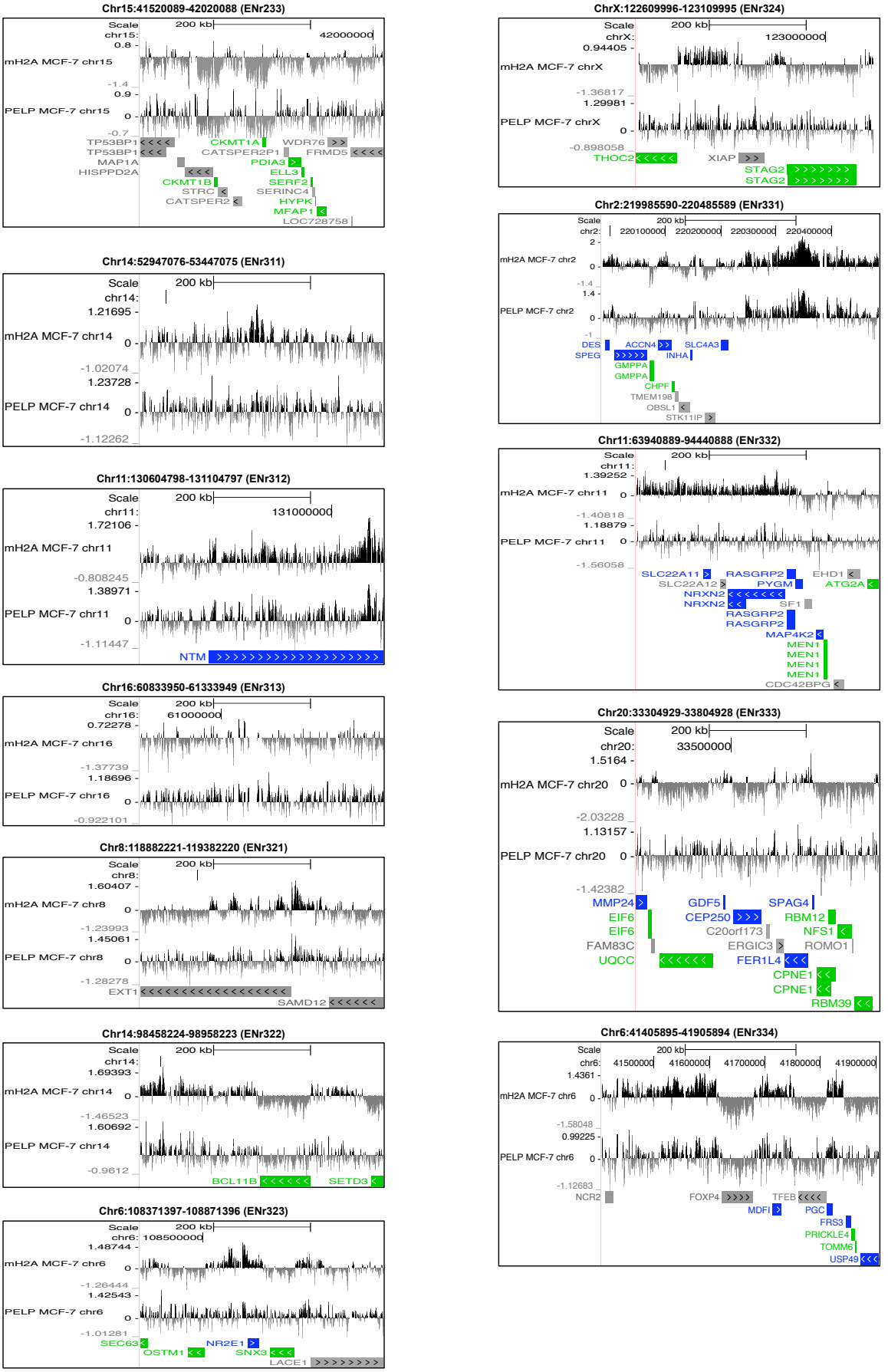

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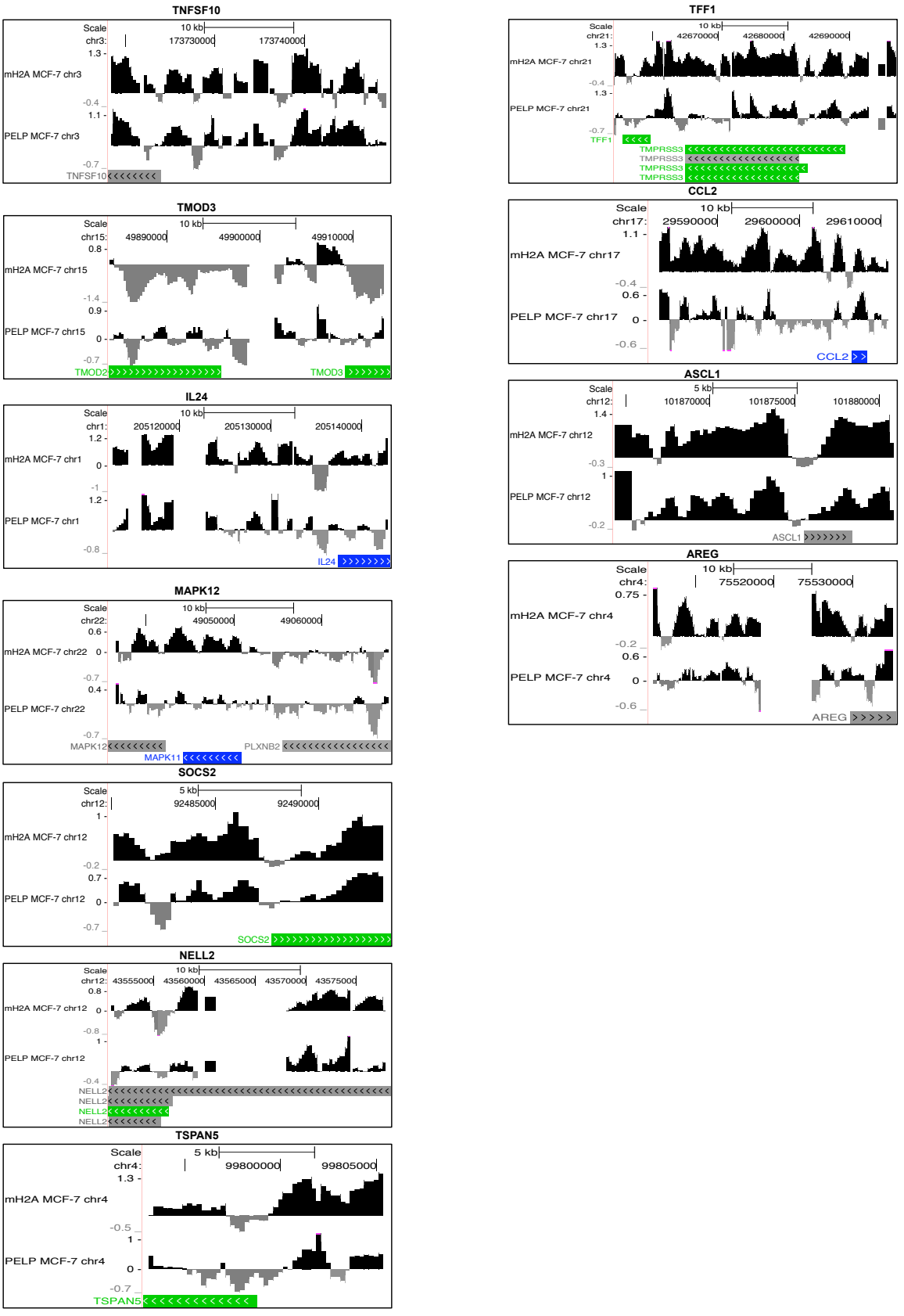

Fig. S6 – Hussey *et al.* (2014)

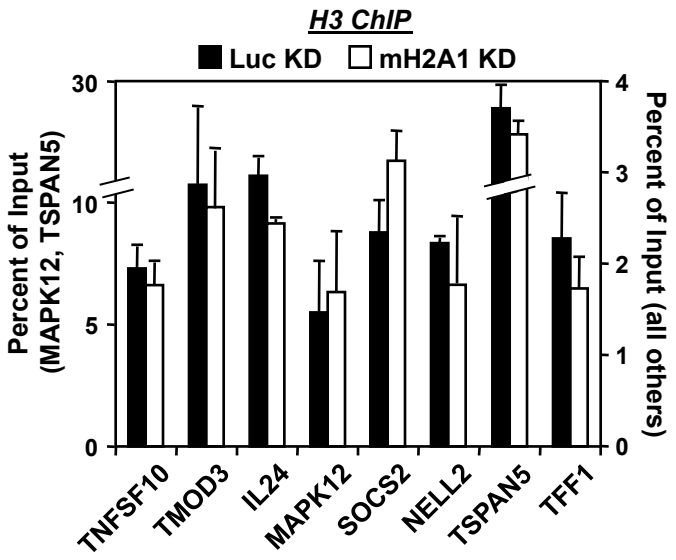

Fig. S7 – Hussey *et al.* (2014)

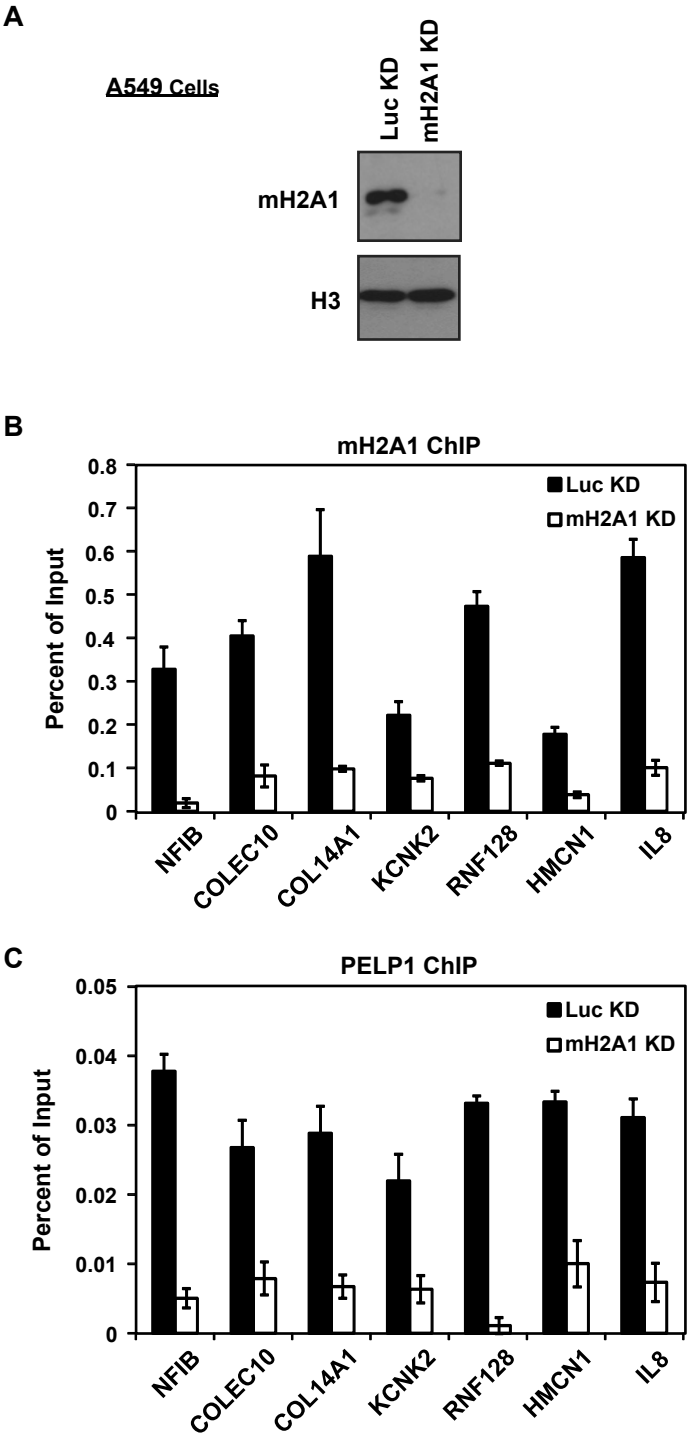

Fig. S8 – Hussey *et al.* (2014)

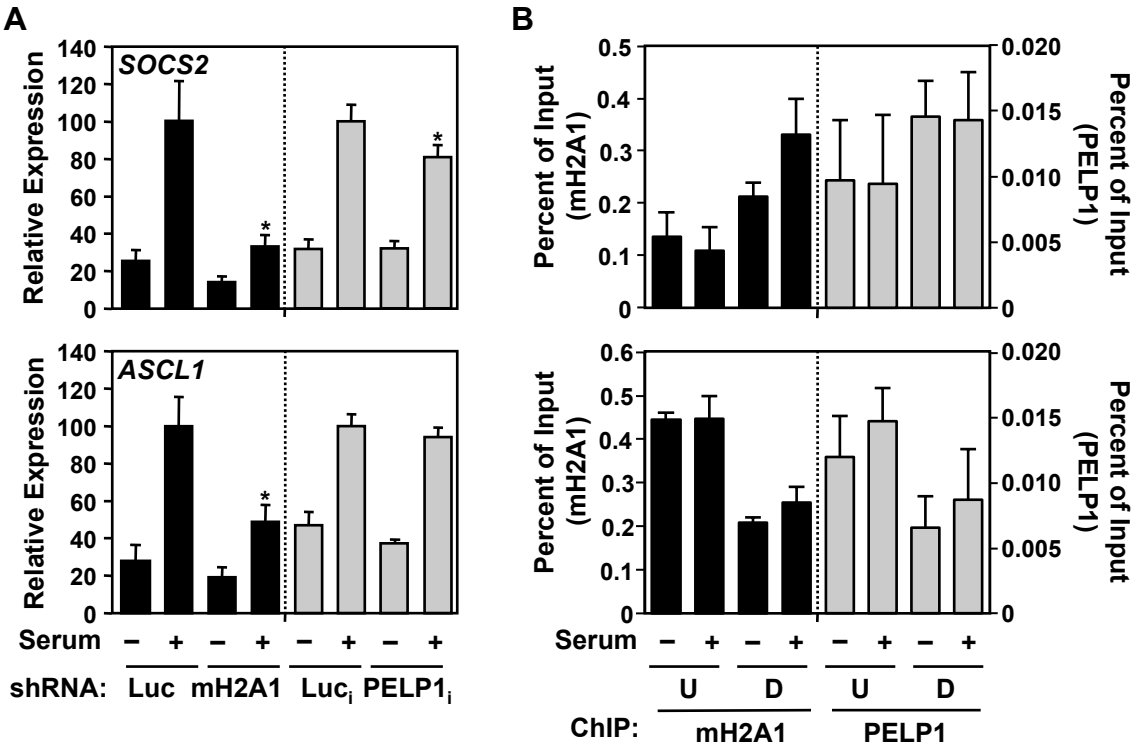

Fig. S9 – Hussey *et al.* (2014)

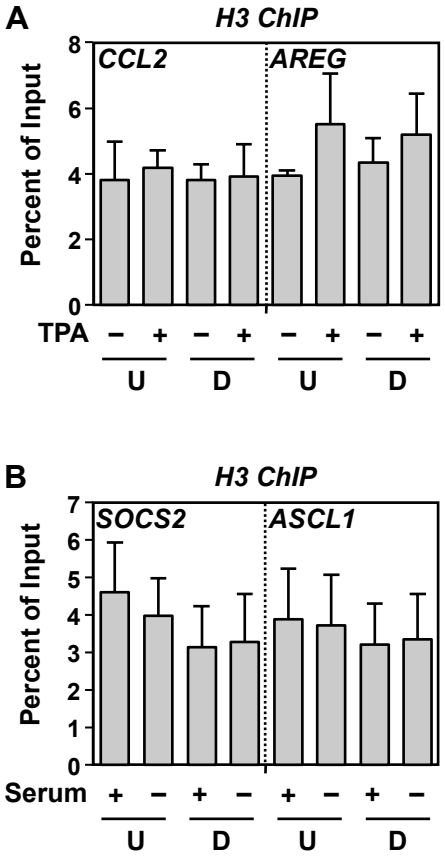

Fig. S10 – Hussey *et al.* (2014)