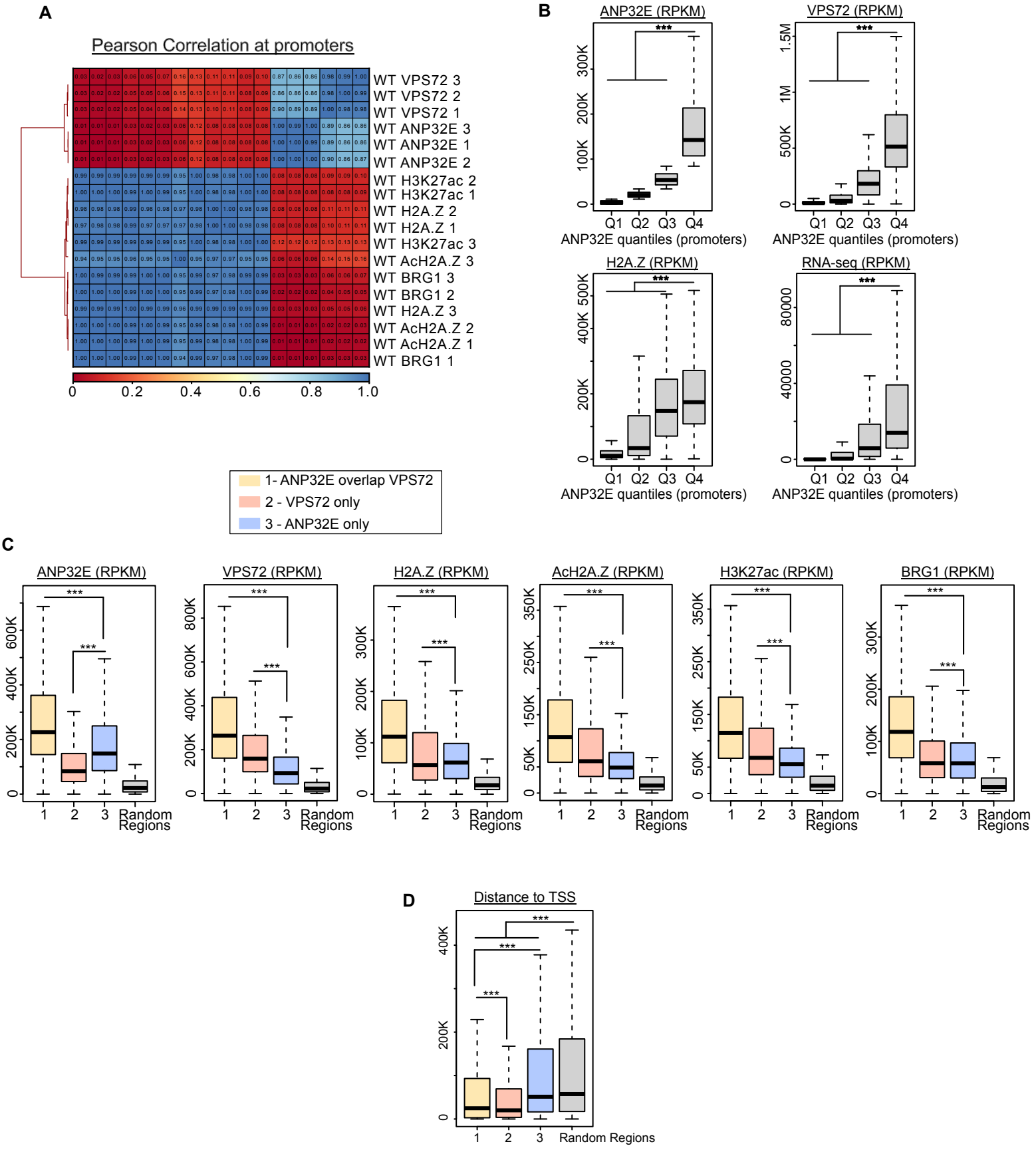

A
Pearson Correlation at promoters
WT VPS72 3
WT VPS72 2
WT VPS72 1
WT ANP32E 3
WT ANP32E 1
WT ANP32E 2
WT H3K27ac 2
WT H3K27ac 1
WT H2A.Z 2
WT H2A.Z 1
WT H3K27ac 3
WT AcH2A.Z 3
WT BRG1 3
WT BRG1 2
WT H2A.Z 3
WT AcH2A.Z 2
WT AcH2A.Z 1
WT BRG1 1
0 0.2 0.4 0.6 0.8 1.0
B
ANP32E (RPKM)
VPS72 (RPKM)
H2A.Z (RPKM)
RNA-seq (RPKM)
Q1 Q2 Q3 Q4
ANP32E quantiles (promoters)
1- ANP32E overlap VPS72
2 - VPS72 only
3 - ANP32E only
C
ANP32E (RPKM)
VPS72 (RPKM)
H2A.Z (RPKM)
AcH2A.Z (RPKM)
H3K27ac (RPKM)
BRG1 (RPKM)
1 2 3 Random Regions
D
Distance to TSS
1 2 3 Random Regions

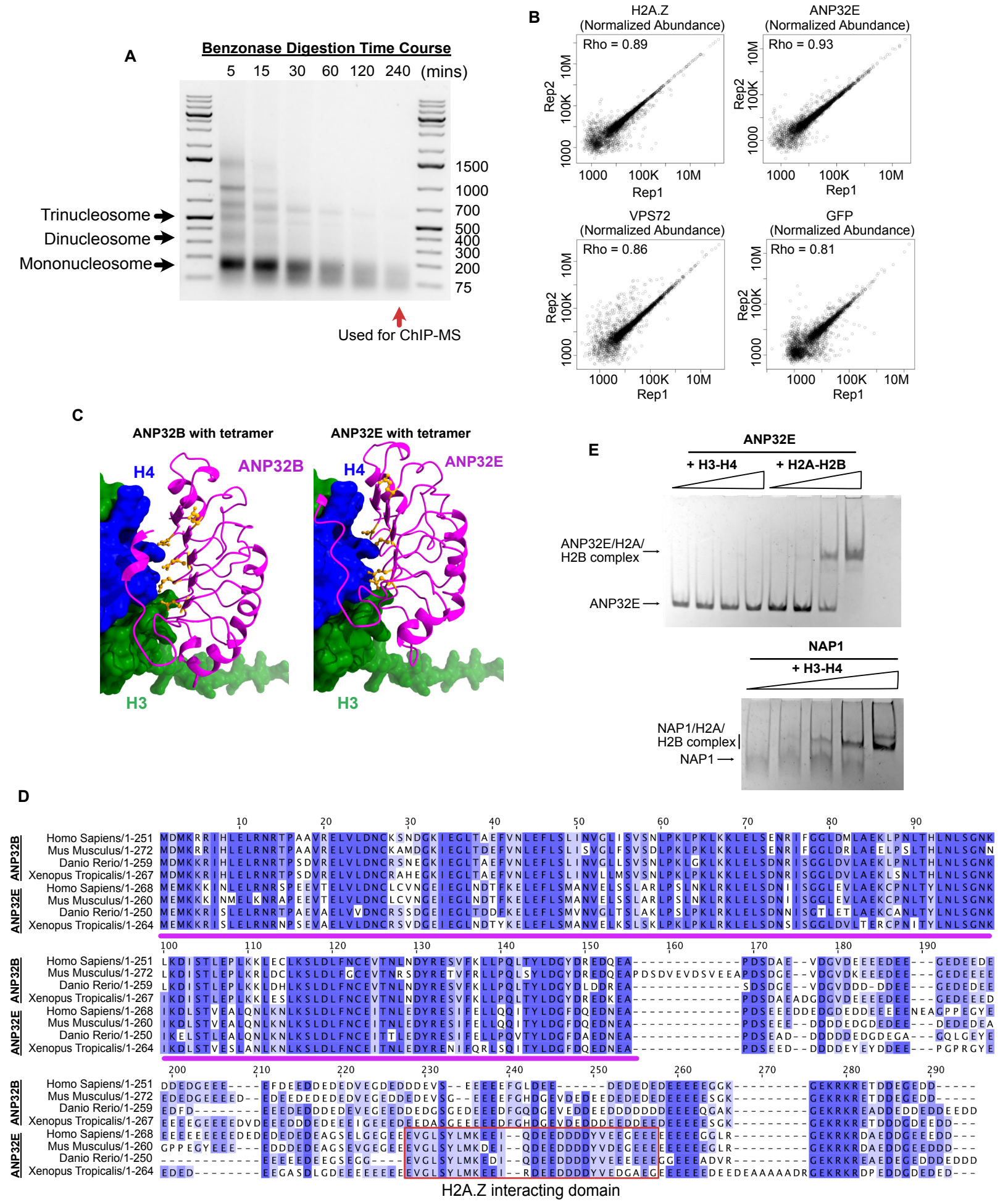
Supplementary Figure 1: The enrichment of ANP32E, VPS72, and H2A.Z are positively correlated with each other

A. Heatmap of Pearson correlation of three replicates for H2A.Z, VPS72, ANP32E, AcH2A.Z, H3K27ac, and BRG1 at promoters in WT MEFs. Pearson correlation values are provided.

B. Boxplots of RPKM enrichment of ANP32E, VPS72, H2A.Z, and RNA-seq across four enrichment quantiles. Quantiles are partitioned based on ANP32E levels at promoters from the lowest signal (Q1) to the highest signal (Q4). One-sided Wilcoxon rank-sum test was used to calculate p-values. ***$p < 0.0001$.

C. Boxplots of RPKM enrichment of ANP32E, VPS72, H2A.Z, AcH2A.Z, H3K27ac, and BRG1 at the peak regions from the intersection between ANP32E and VPS72 bound loci, and their individual occupied regions. ANP32E only = blue; ANP32E and VPS72 = orange; VPS72 only = pink. One-sided Wilcoxon rank-sum test was used to calculate p-values. ***$p < 0.0001$.

D. Boxplot of distance from peaks to nearest TSS with regions broken up according to enrichment of ANP32E and/or VPS72. ANP32E only, blue; ANP32E over VPS72, orange; VPS72 only, pink. One-sided Wilcoxon rank-sum test was used to calculate p-values. ***$p < 0.0001$.


A
Benzonase Digestion Time Course
5 15 30 60 120 240 (mins)
1500
1000
700
500
400
300
200
75
Trinucleosome
Dinucleosome
Mononucleosome
Used for ChIP-MS
B
H2A.Z (Normalized Abundance)
Rho = 0.89
ANP32E (Normalized Abundance)
Rho = 0.93
VPS72 (Normalized Abundance)
Rho = 0.86
GFP (Normalized Abundance)
Rho = 0.81
Rep1
Rep2
1000
100K
10M
C
ANP32B with tetramer
ANP32E with tetramer
H4
ANP32B
ANP32E
H3
E
ANP32E
+ H3-H4
+ H2A-H2B
ANP32E/H2A/H2B complex
ANP32E
NAP1
+ H3-H4
NAP1/H2A/H2B complex
NAP1
D
ANP32B
ANP32E
Homo Sapiens/1-251
Mus Musculus/1-272
Danio Rerio/1-259
Xenopus Tropicalis/1-267
Homo Sapiens/1-268
Mus Musculus/1-260
Danio Rerio/1-250
Xenopus Tropicalis/1-264
H2A.Z interacting domain

Supplementary Figure 2: The N-terminal of ANP32E is predicated to interact with H3-H4 tetramers as ANP32B

A. Gel electrophoresis of the length of DNA fragments digested by Benzonase at different time points for ChIP-MS. Mononucleosomes = ~150bp fragment length; dinucleosomes = ~300bp fragment length; trinucleosome = ~500bp fragment length.

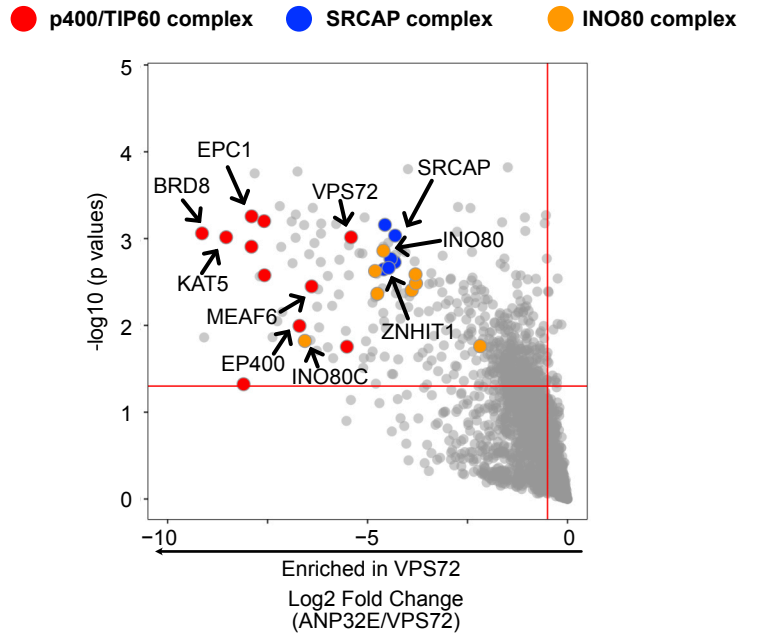
B. Scatterplot depicts enrichment for two replicates of H2A.Z ChIP-MS, ANP32E ChIP-MS, VPS73 ChIP-MS and GFP ChIP-MS. Positive Rho values indicate a positive correlation between replicates.

C. AlphaFold3 predicted structure of ANP32B bound with H3-H4 tetramers and ANP32E bound with H3-H4 tetramers. The human protein sequences were used for predictions.

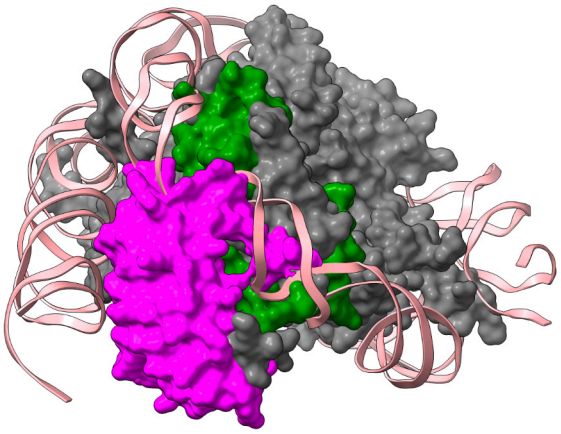
D. Protein alignment of ANP32B and ANP32E in Homo Sapiens, Mus Musculus, Danio Rerio, and Xenopus Tropicalis. The magenta lines highlighted the evolutionarily conserved N-terminal (1-155), and the square highlights the conserved H2A.Z interacting domain (ZID) at the C-terminus of ANP32E.

E. EMSA assay investigating the interaction between ANP32E with H3-H4 tetramers or H2-H2B dimers. NAP1 is included a positive control for H3-H4 tetramer interaction.

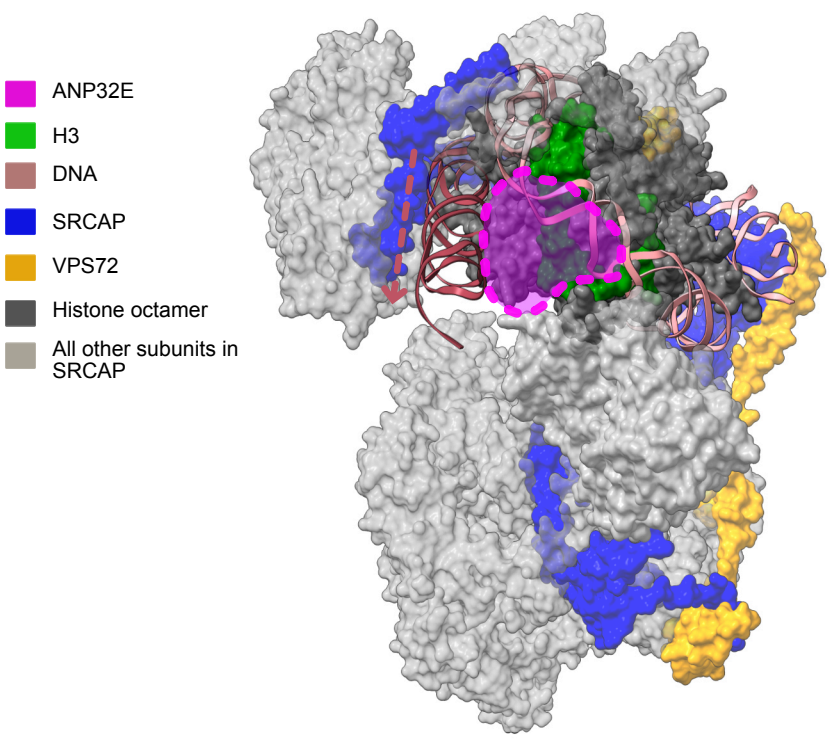
A


p400/TIP60 complex
SRCAP complex
INO80 complex
-log10 (p values)
5
4
3
2
1
0
EPC1
BRD8
VPS72
SRCAP
INO80
KAT5
MEAF6
ZNHIT1
EP400
INO80C
−10
−5
0
Enriched in VPS72
Log2 Fold Change
(ANP32E/VPS72)

B



C


ANP32E
H3
DNA
SRCAP
VPS72
Histone octamer
All other subunits in SRCAP

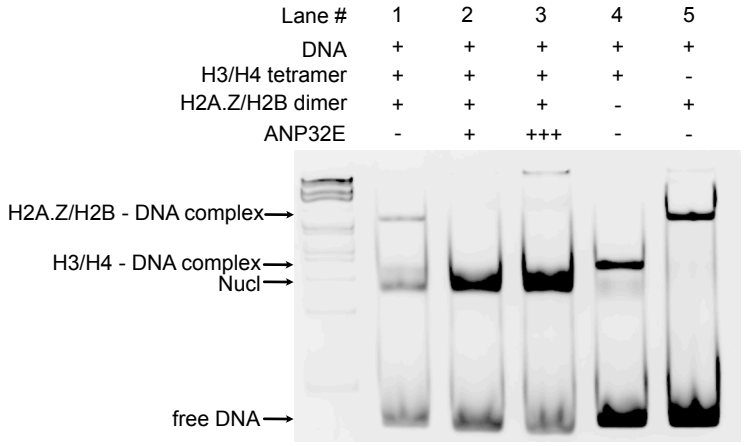
Supplementary Figure 3: ANP32E is predicated to interact with histone octamers and stabilizes partial unwrapping nucleosomes within SRCAP complex

A. Volcano plot of differential interacting proteins comparing the ANP32E ChIP-MS assay against the VPS72 ChIP-MS assay. Only proteins significantly enriched in VPS72 ChIP-MS are shown (Log2FC < 0.5 and p values < 0.05). Major subunits of the p400/TIP60 complex, SRCAP complex, and INO80 complex are labeled in red, blue, and orange, respectively.

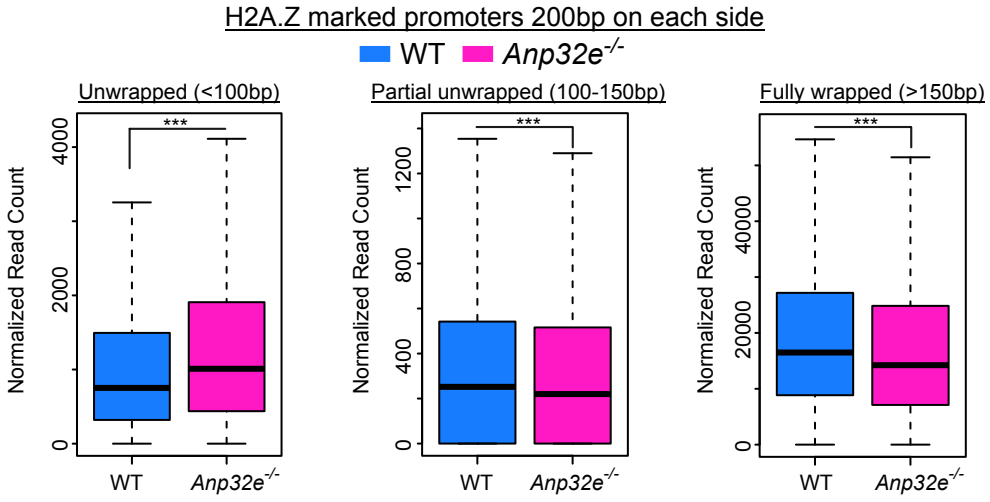
B. AlphaFold3 predicted structure of ANP32E bound with partial unwrapped nucleosome. The color of each protein was labeled as follows: H3 - green; ANP32E – magenta.

C. Re-analysis of nucleosome structures when bound by the published active state of the SRCAP remodeling complex, such that AlphaFold3 predicted N-terminal of ANP32E structure has been aligned and overlaid. The transparent magenta dotted lines represent the location where ANP32E binding occurs, based on panel B. DNA is partially unwrapped from nucleosomes when SRCAP is in the active state, and an arrow depicts the location of unwrapped DNA exiting the nucleosome.

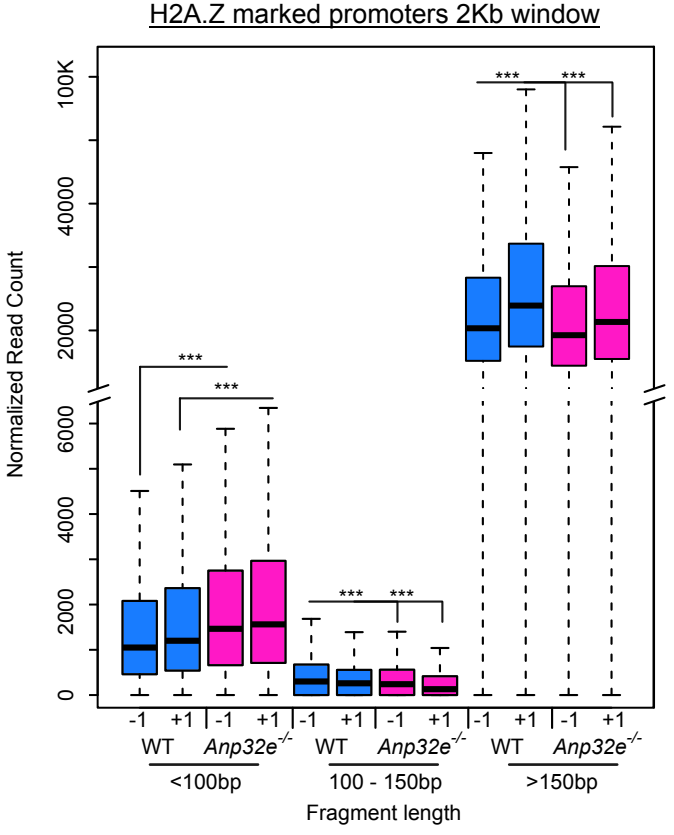
A


Lane # 1 2 3 4 5
DNA + + + + +
H3/H4 tetramer + + + + -
H2A.Z/H2B dimer + + + - +
ANP32E - + +++ - -
H2A.Z/H2B - DNA complex
H3/H4 - DNA complex
Nucl
free DNA

B


H2A.Z marked promoters 200bp on each side
WT Anp32e-/-
Unwrapped (<100bp)
Partial unwrapped (100-150bp)
Fully wrapped (>150bp)
Normalized Read Count
***
WT
Anp32e-/-

C

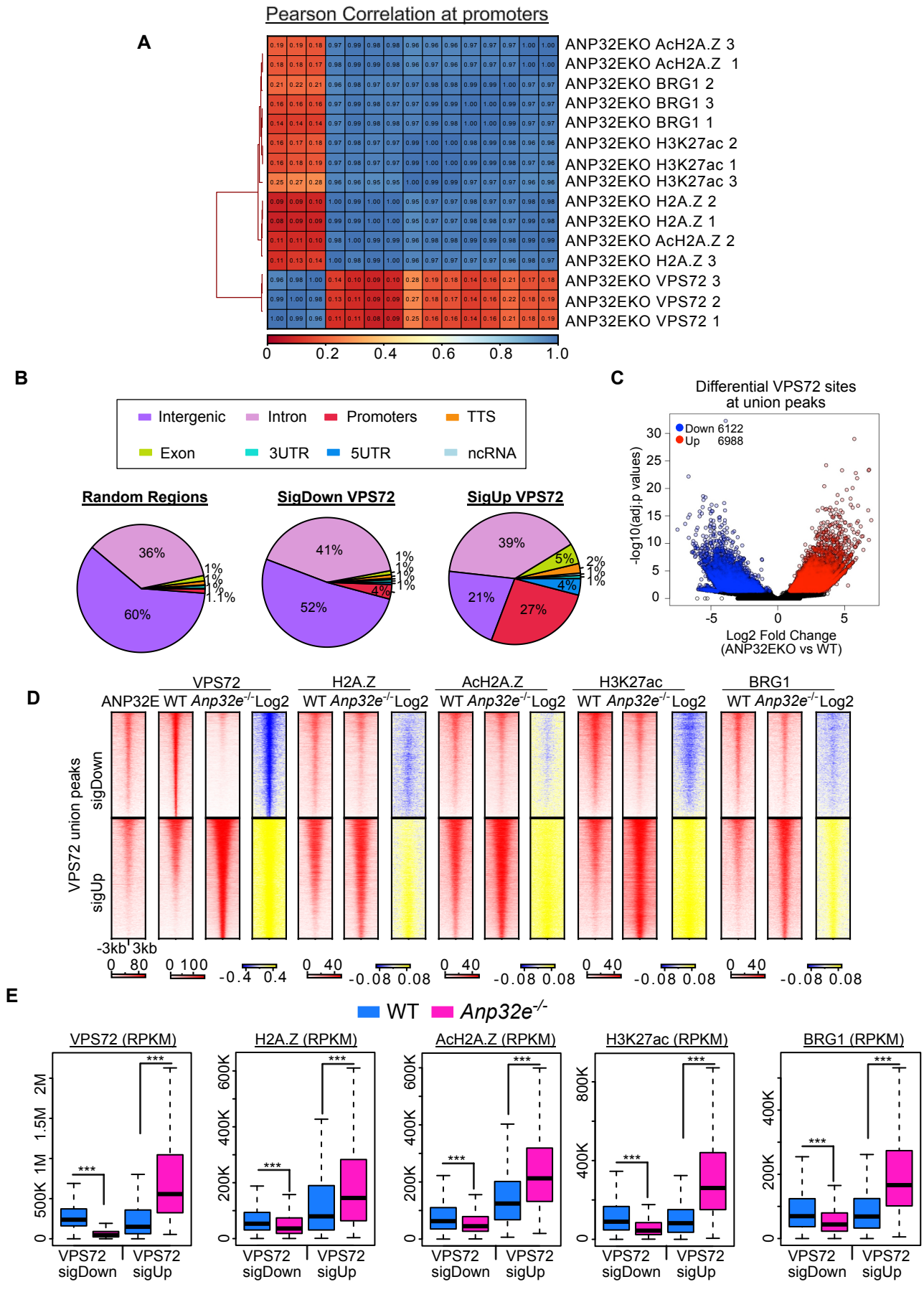

H2A.Z marked promoters 2Kb window
Normalized Read Count
***
-1 +1 -1 +1 -1 +1 -1 +1 -1 +1 -1 +1
WT Anp32e-/- WT Anp32e-/- WT Anp32e-/-
<100bp
100 - 150bp
>150bp
Fragment length

Supplementary Figure 4: Loss of ANP32E caused more unwrapped nucleosomes at downstream of the TSS

A. H2A.Z nucleosome assembly assays in which DNA is incubated with H3/H4 tetramers and H2A.Z/H2B dimers, along with different amounts of ANP32E.

B. Boxplots of normalized read count for DNA fragments wrapped around nucleosomes at H2A.Z-marked promoters (200bp on each side of TSS) in WT and ANP32E null MEFs. Unwrapped = less than 100bp; partial unwrapped = 100 to 150bp; fully wrapped = greater than 150bp. One-sided Wilcoxon rank-sum test was used to calculate p-values. ***$p < 0.0001$.

C. Boxplots of normalized read count of nucleosome wrapping at downstream (+1 Kb) and upstream (-1 Kb) TSS positions in WT and ANP32E null MEFs. One-sided Wilcoxon rank-sum test was used to calculate p-values and show their significance. ***$p < 0.0001$.


Pearson Correlation at promoters
A
ANP32EKO AcH2A.Z 3
ANP32EKO AcH2A.Z 1
ANP32EKO BRG1 2
ANP32EKO BRG1 3
ANP32EKO BRG1 1
ANP32EKO H3K27ac 2
ANP32EKO H3K27ac 1
ANP32EKO H3K27ac 3
ANP32EKO H2A.Z 2
ANP32EKO H2A.Z 1
ANP32EKO AcH2A.Z 2
ANP32EKO H2A.Z 3
ANP32EKO VPS72 3
ANP32EKO VPS72 2
ANP32EKO VPS72 1
0 0.2 0.4 0.6 0.8 1.0
B
Intergenic
Intron
Promoters
TTS
Exon
3UTR
5UTR
ncRNA
Random Regions
SigDown VPS72
SigUp VPS72
36%
60%
41%
52%
39%
21%
27%
5%
4%
C
Differential VPS72 sites at union peaks
Down 6122
Up 6988
-log10(adj.p values)
Log2 Fold Change (ANP32EKO vs WT)
D
VPS72
H2A.Z
AcH2A.Z
H3K27ac
BRG1
ANP32E WT Anp32e-/- Log2
VPS72 union peaks
sigDown
sigUp
-3kb 3kb
E
WT
Anp32e-/-
VPS72 (RPKM)
H2A.Z (RPKM)
AcH2A.Z (RPKM)
H3K27ac (RPKM)
BRG1 (RPKM)
VPS72 sigDown
VPS72 sigUp

Supplmentary Figure 5: Loss of ANP32E led to increased VPS72, H2A.Z, histone acetylation, and BRG1 binding at VPS72 union peaks

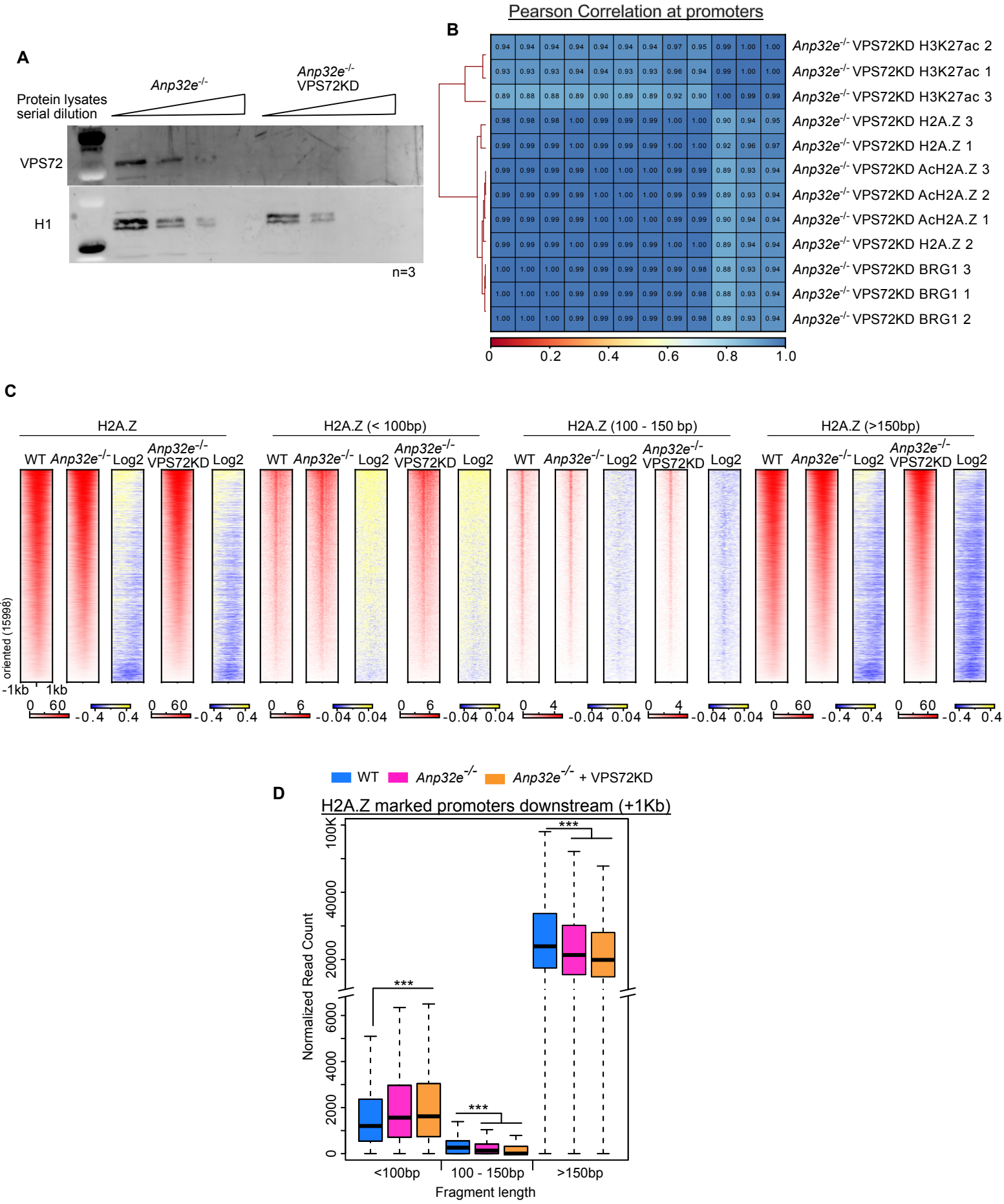
A. Heatmap of Pearson correlations for three replicates for H2A.Z, VPS72, ANP32E, AcH2A.Z, H3K27ac, and BRG1 CUT&Tag at promoters in ANP32E null MEFs. The Pearson correlation values are provided.

B. Pie chart of genomic distribution of increased VPS72 sites and decreased VPS72 sites compared with random regions (55,000 random regions with 250bp fragment length).

C. Volcano plot of differential VPS72 binding in ANP32E null MEFs versus WT MEFs at VPS72 union peaks. Diffbind was used to identify differential binding sites. Increased VPS72 sites (6988 sites) are shown in red (log2FC > 0.5 and p. Adjusted values < 0.05). Decreased VPS72 sites (6122 sites) are shown in blue (log2FC < 0.5 and p. Adjusted values < 0.05).

D. Heatmap of ANP32E, VPS72, H2A.Z, AcH2A.Z, H3K27ac, and BRG1 enrichment in WT MEFs and ANP32E null MEFs at increased VPS72 sites (peaks) and decreased VPS72 sites (peaks). Log2 is calculated for the RPKM values in ANP32E null MEFs over WT MEFs.

E. Boxplot of RPKM normalized VPS72, H2A.Z, AcH2A.Z, H3K27ac, and BRG1 enrichment at increased VPS72 sites (peaks) and decreased VPS72 sites (peaks) in WT MEFs and ANP32E null MEFs. WT MEFs = blue; ANP32E null MEFs = magenta. One-sided Wilcoxon rank-sum test was used to calculate p-values. ***p < 0.0001.


A
Anp32e-/-
Anp32e-/- VPS72KD
Protein lysates serial dilution
VPS72
H1
n=3
B
Pearson Correlation at promoters
Anp32e-/- VPS72KD H3K27ac 2
Anp32e-/- VPS72KD H3K27ac 1
Anp32e-/- VPS72KD H3K27ac 3
Anp32e-/- VPS72KD H2A.Z 3
Anp32e-/- VPS72KD H2A.Z 1
Anp32e-/- VPS72KD AcH2A.Z 3
Anp32e-/- VPS72KD AcH2A.Z 2
Anp32e-/- VPS72KD AcH2A.Z 1
Anp32e-/- VPS72KD H2A.Z 2
Anp32e-/- VPS72KD BRG1 3
Anp32e-/- VPS72KD BRG1 1
Anp32e-/- VPS72KD BRG1 2
0 0.2 0.4 0.6 0.8 1.0
C
H2A.Z
H2A.Z (< 100bp)
H2A.Z (100 - 150 bp)
H2A.Z (>150bp)
WT Anp32e-/- Log2 Anp32e-/- VPS72KD Log2
H2A.Z marked promoters oriented (15998)
-1kb 1kb
D
WT Anp32e-/- Anp32e-/- + VPS72KD
H2A.Z marked promoters downstream (+1Kb)
Normalized Read Count
0 2000 4000 6000 20000 40000 100K
***
<100bp 100 - 150bp >150bp
Fragment length

Supplementary Figure 6: Accumulation of VPS72 in the absence of ANP32E generates hyperaccessible regions at H2A.Z - marked promoters

A. Western blot of VPS72 knockdown in ANP32E null MEFs. ANP32E null MEFs were treated with non-targeting siRNA (left) and VPS72 siRNA (right). Serial dilution of protein lysate concentration (stock, ½. ¼, ⅛) was loaded. The size of the VPS72 protein is 57 kDa, and the H1 control protein is 31 kDa.

B. Heatmap of Pearson correlations for three replicates for H2A.Z, VPS72, ANP32E, AcH2A.Z, H3K27ac, and BRG1 CUT&Tag at promoters in VPS72 knockdown in ANP32E null MEFs. The Pearson correlation values are provided.

C. Heatmap of normalized read count for DNA fragments wrapped around nucleosomes at H2A.Z-marked promoters (TSS ± 1kb). Unwrapped = less than 100bp; partial unwrapped = 100 to 150bp; fully wrapped = greater than 150bp. For each nucleosome wrapping status, the first log2 fold change depicts change of ANP32E null MEFs compared with WT MEFs, and the second log2 fold change depicts change of VPS72 knockdown in ANP32E null MEFs compared with WT MEFs.

D. Boxplot of normalized read count for nucleosome wrapping at loci downstream from TSS (+1 Kb) in WT MEFs, ANP32E null MEFs, and VPS72KD with ANP32E null MEFs. A one- tailed Wilcoxon rank sum test was used to calculate p-values.
***$p < 0.0001$.