Supporting Online Material

Foxa2 and H2A.Z Mediate Nucleosome Depletion and Embryonic Stem Cell Differentiation

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This supplement contains:

Table S1, related to Figures 1 and 2

Figure Legends for Supplemental Figures S1 to S5

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Figure S1. Related to Figure 1

(A) Schematic view of Nucleosome mapping via high-throughput sequencing. **(B)** An example of DANPOS calling nucleosome positions and dynamic regions.

(B)

Figure S2. Related to Figure 2

Nucleosome dynamics and gene regulation. (**A**) Nucleosome distribution surrounding TSS of active and silent genes in ES and EHP cells. Top 1,000 highly expressed and 1,000 silent genes in undifferentiated and differentiated ES cells were selected from gene expression profiles by microarray analysis. (**B)** Distributions of H2A.Z,H2A.X and Foxa2 near TSS. (**C**) Percentage distribution of H2A.Z, H2A.X and Foxa2 in the mouse genome. (**D**) Genome-wide co-location analysis of Foxa2 and H2A.Z obtained by mutual plotting the distribution of two binding sites. Note the striking co-occupancy of the two factors. (**E**) Co-localizations of Foxa2 and H2A.Z at nucleosome depletion regions by sequential ChIPs in both orders. (**F**) Percentage distribution of Foxa2/H2A.Z-enriched nucleosome depletion regions to the mouse genome. Genome, mouse whole genome (mm8); NucDep, Foxa2/H2A.Z-enriched nucleosome depletion regions. (**G** and **H**) Genome-wide distribution of partial nucleosome depletion or partial occupation regions near H2A.Z, H2A.X and Foxa2 binding sites. Error bars represent standard error of the mean.

Figure S3. Related to Figure 3

(A) Targeted differentiation of ES cells into EHP cells. (B) Suppresion of gene expression by siRNA for Foxa2, H2afz (H2A.Z), Smarca4 (Brg1), Kat5 (Tip60) or Nap1l1 in ES cells was shown by western blotting. Actin was used as loading controls. (**C**) Co-transfection and expression of Foxa2 and eGFP in ES cells. (**D**) Nucleosome occupancy by qPCR at nucleosome depletion regions in control ES cells and ES cells overexpressing Foxa2. ES, ES cells transfected with eGFP expression vectors; ES + Foxa2, ES cells transfected with both eGFP and Foxa2 expression vectors. (**E**) Nucleosome occupancy in sorted Foxa2^{+}ENDM1^{-} cells. Error bars represent standard error of the mean.

Figure S4. Related to Figure 4

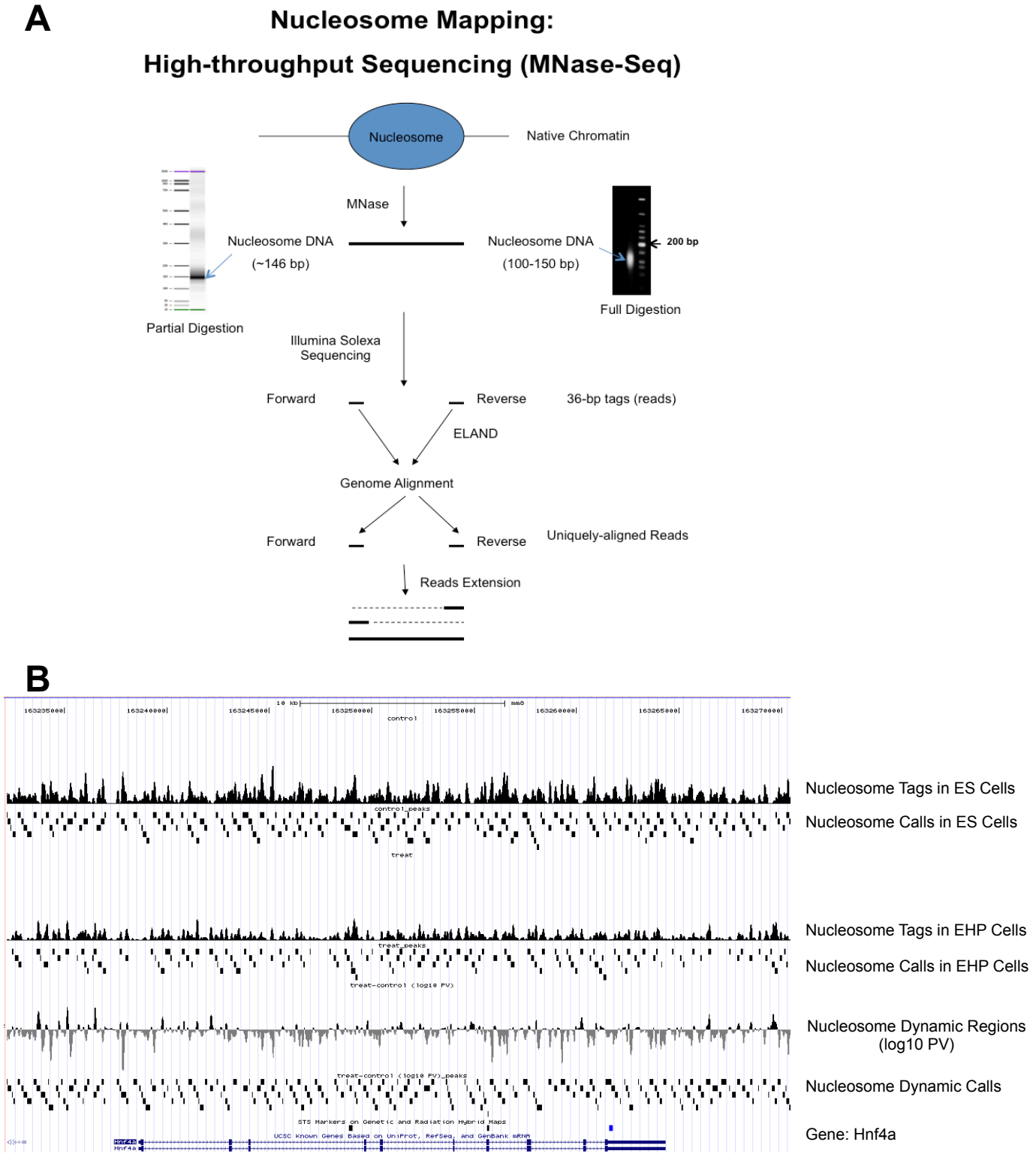
ChIP assays for twelve markers of chromatin remodeling complexes. (**A**) Nap1l1, Smarca4 and Kat5 are bound at nucleosome depletion regions during ES cell differentiation. No enrichment of Smarca5 Mbd3 Bptf Asf1a/b Supt6h (Spt6) Ssrp1 Chaf1a (CAF1) Smarca5, Mbd3, Bptf, b, Spt6), Ssrp1, CAF1), Ncl(C23) was found at nucleosome depletion regions by ChIP-qPCR. (**B**) None of these twelve factors was found enriched at those nucleosome occupation regions . (**C**) ChIP assays for Smarca4 (Brg1), Kat5 (Tip60) and Nap1l1 after RNAi to Foxa2, H2afz or both Foxa2 and H2afz. (**D**) ChIP assays for Smarca4 (Brg1), Kat5 (Tip60), Nap1l1, Mbd3, SNF2h and Asf1 at nucleosome depletion regions after the suppression of Smarca4, Kat5 or Nap1l1 by RNAi during ES cell differentiation. Fold =1 for input. Error bars represent standard error of the mean.

Figure S5. Related to Figure 5

Nucleosome dynamics and ES cell differentiation. Flow cytometry analysis with dual cell surface markers of Foxa2/and for the assessment of the extent of differentiation. (**A-D**) ES cells treated with siRNA to both Foxa2 and H2afz (**A**), Smarca4 (**B**), Kat5 (**C**), or Nap1l1 (**D**) show decreased differentiation potential. (**E**) Cell viability counted by FACS after RNAi to Foxa2, H2afz, Foxa2/H2afz, Smarca4, Kat5, Nap1l1, or RG108 treatment. (**F**) Nucleosme occupancy at these regions after RNAi to Foxa2, H2afz, Foxa2/H2afz, Smarca4, Kat5, or Nap1l1. EHP, sorted EHP cells. Error bars represent standard error of the mean.

Supplemental Figure

Click here to download Supplemental Figure: es cell_CellStemCell_suppl_06252012.pdf



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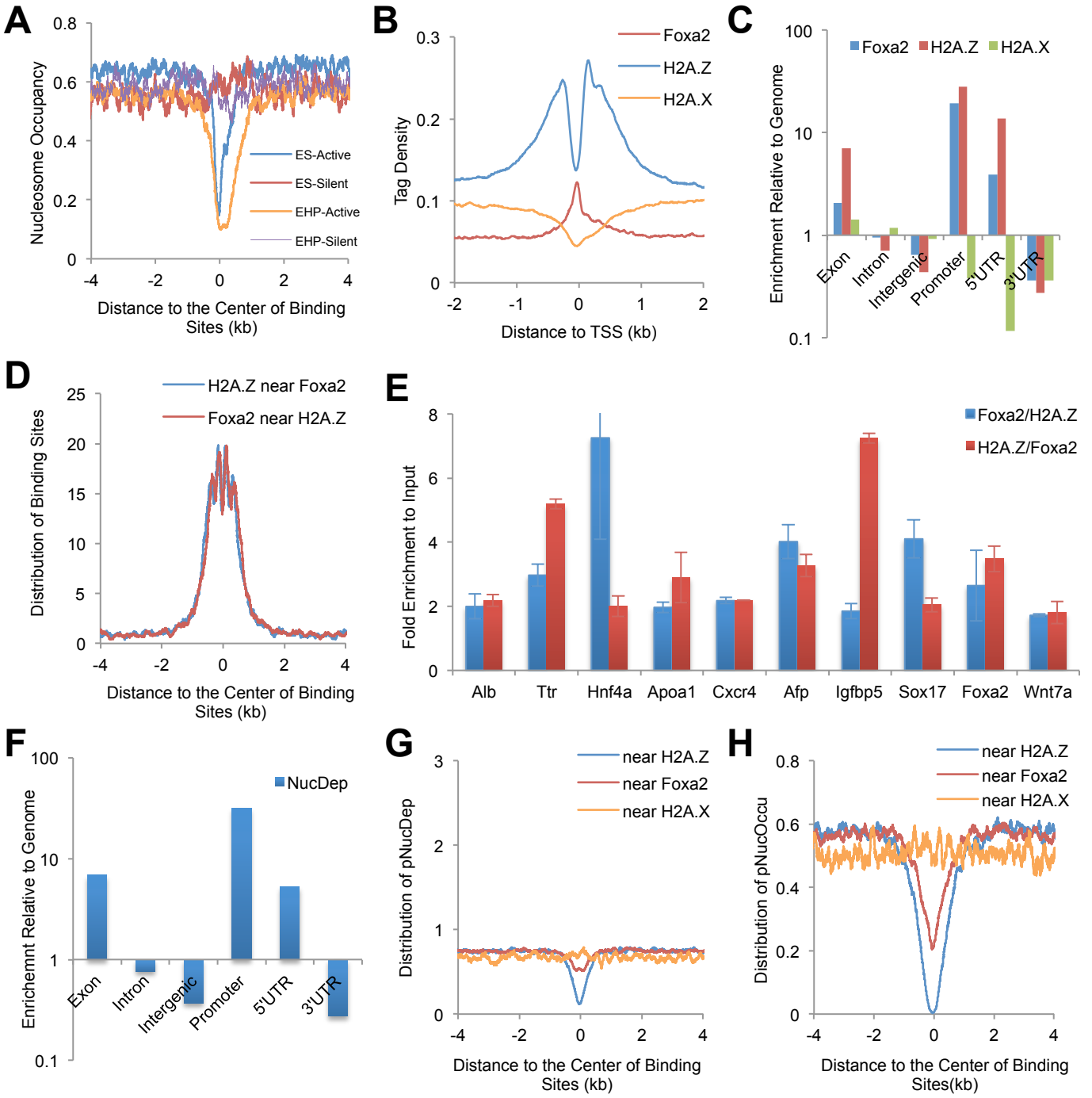

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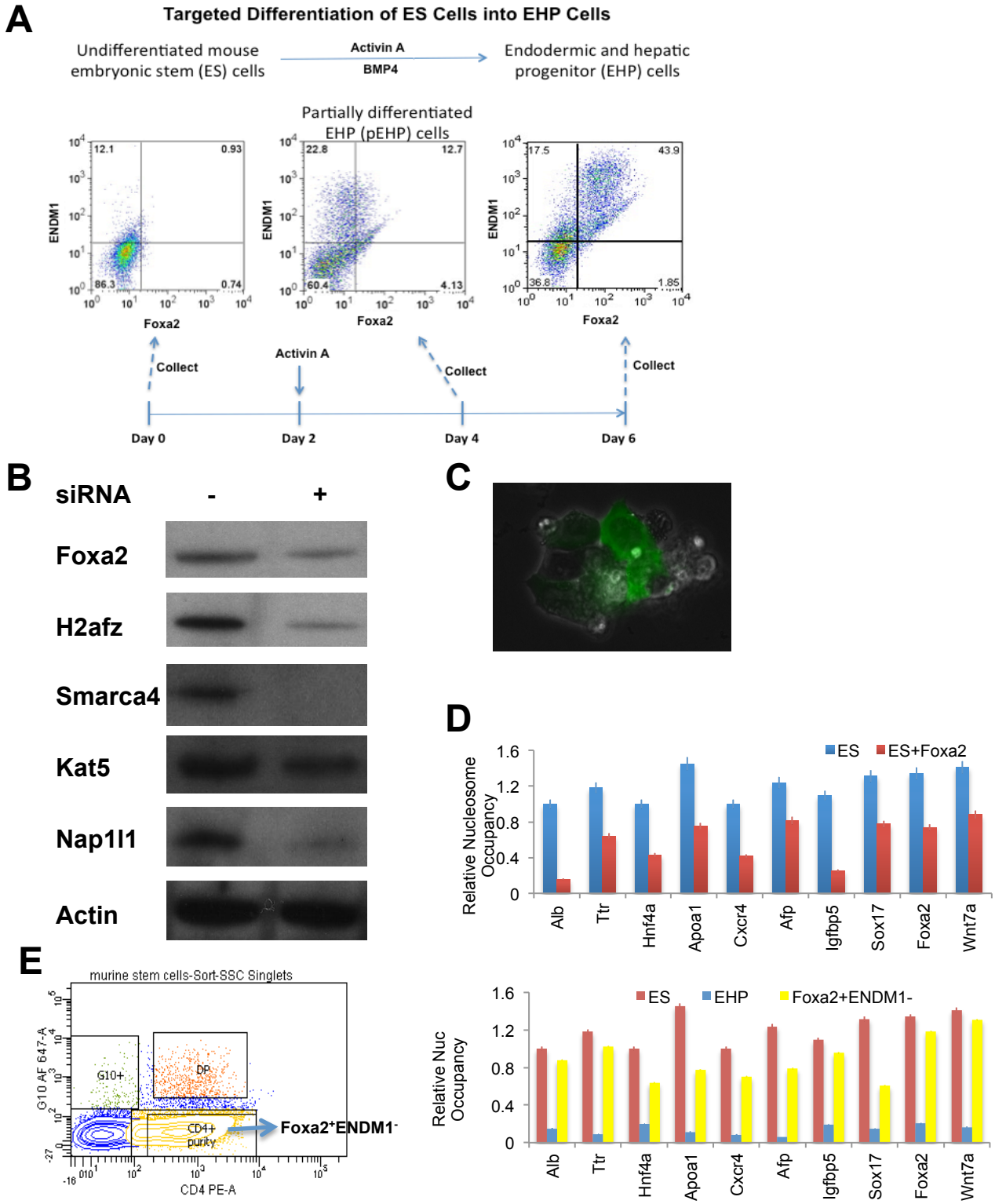

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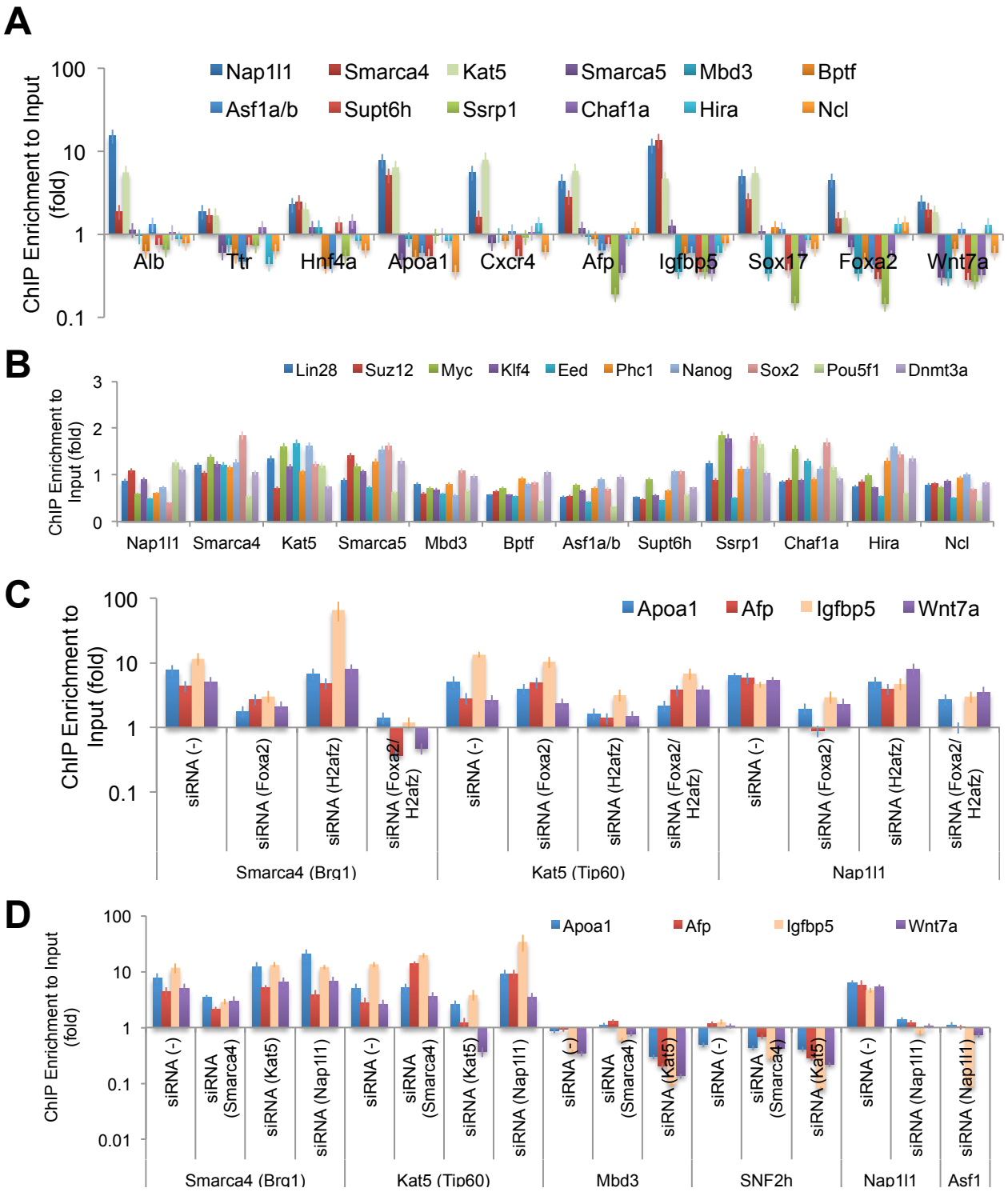

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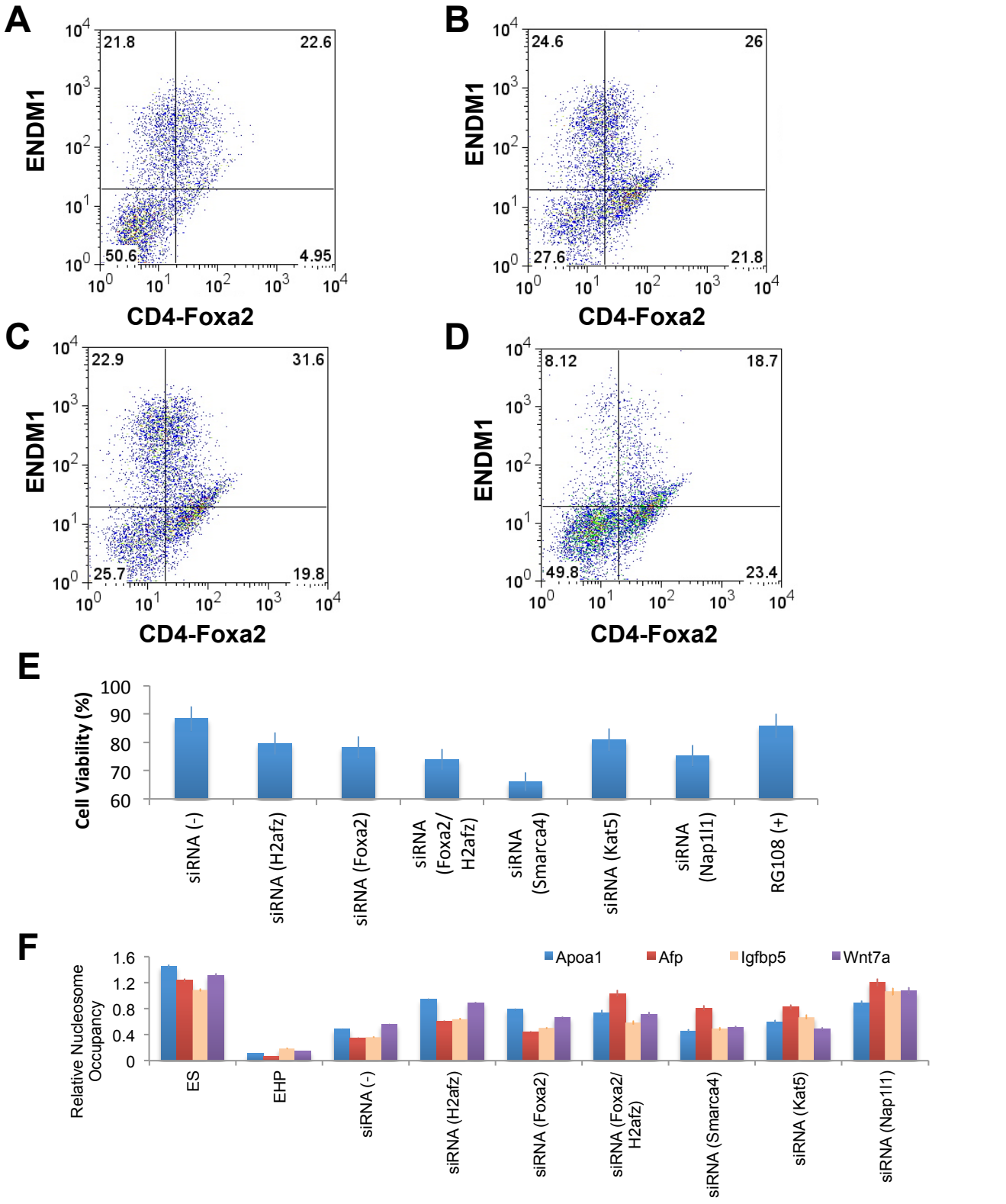

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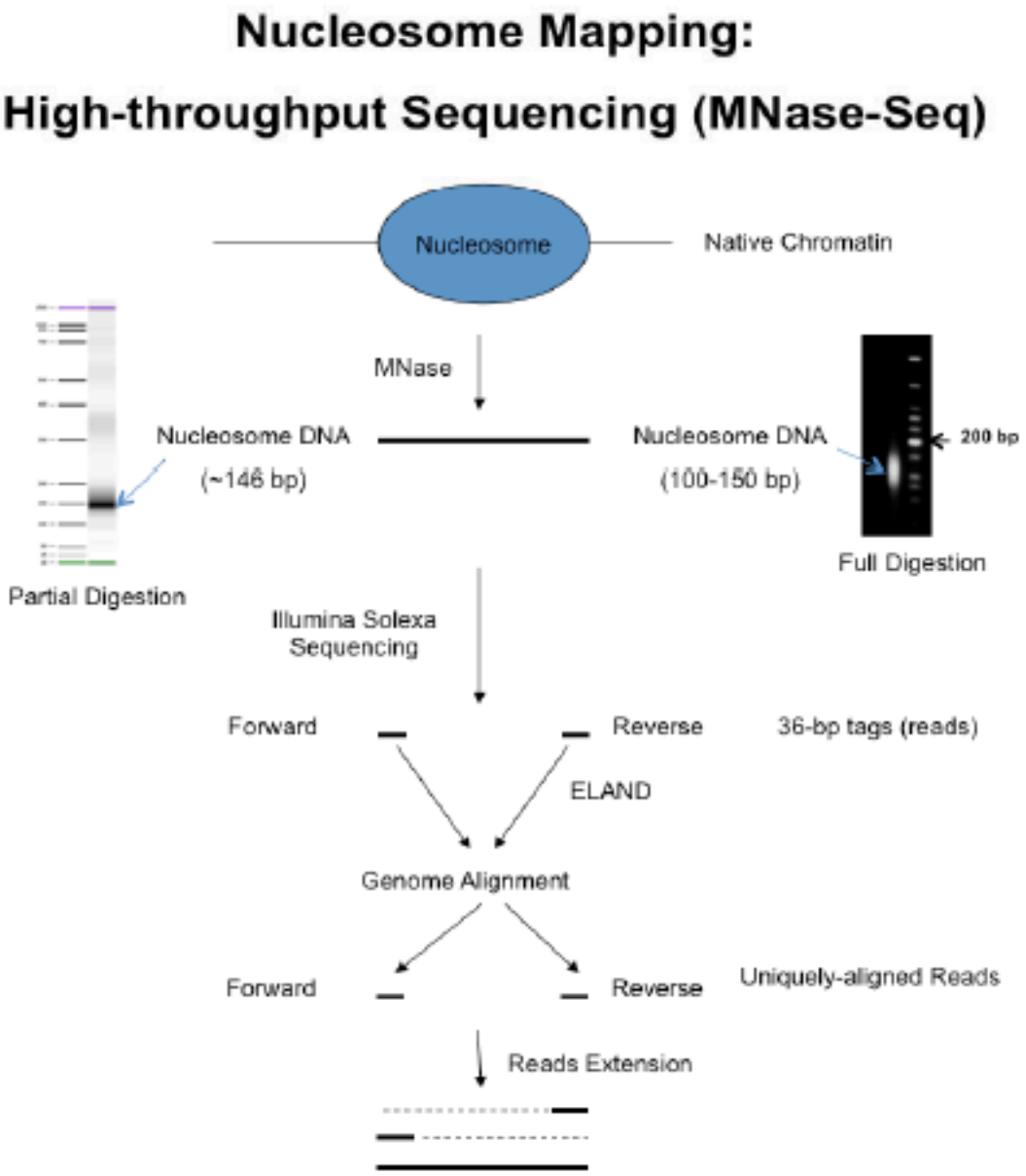
Supplemental Figure

A



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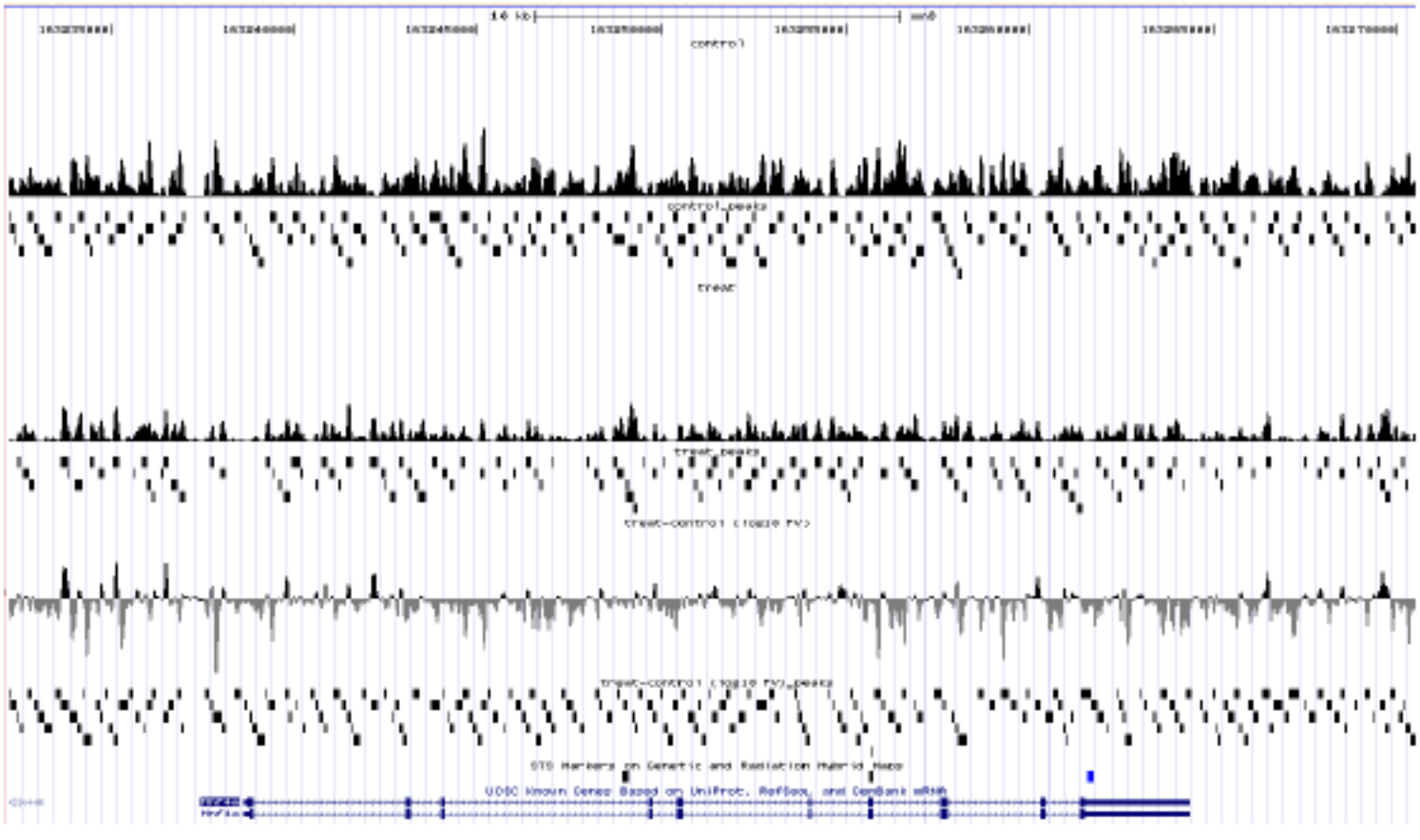

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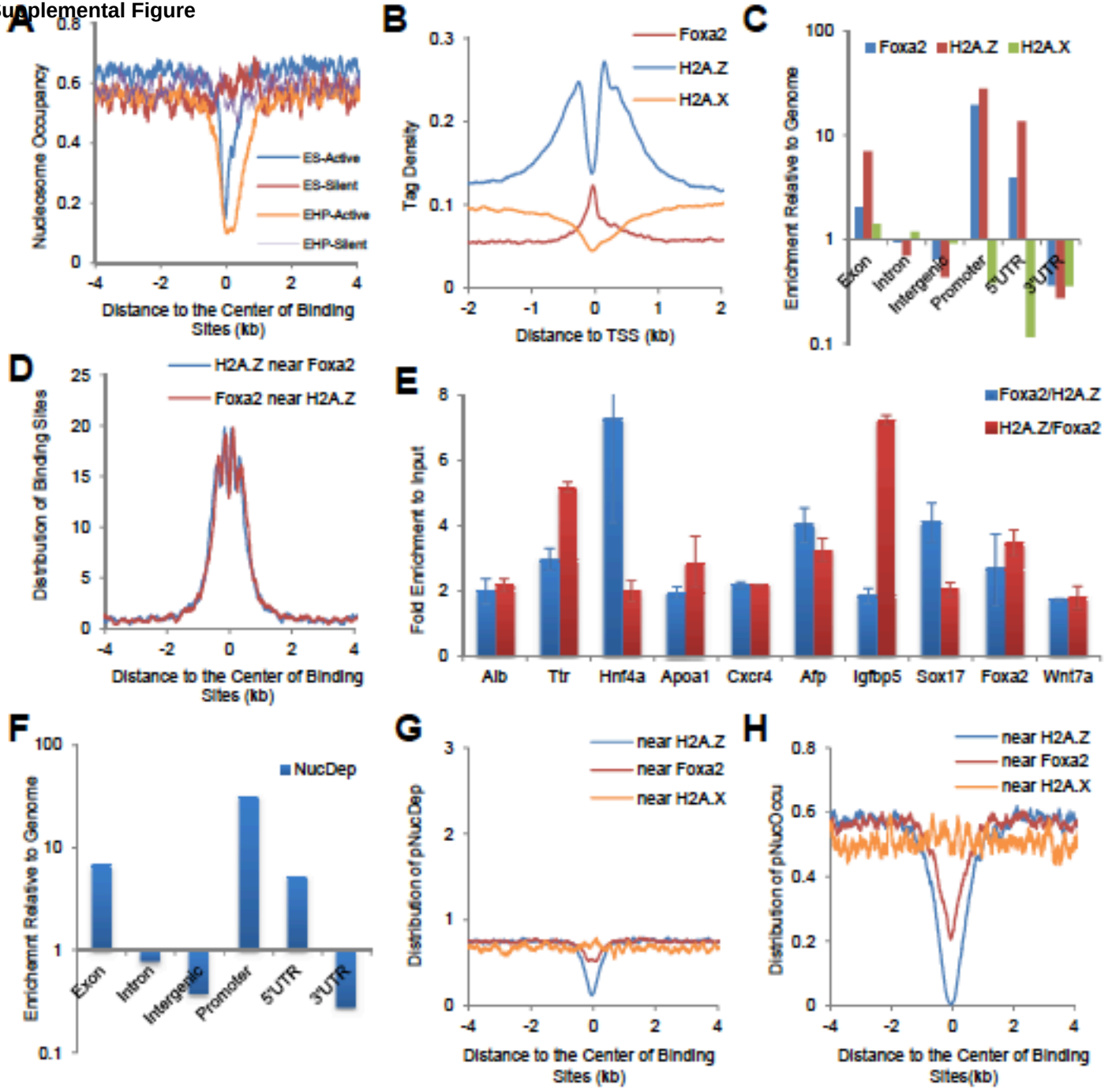

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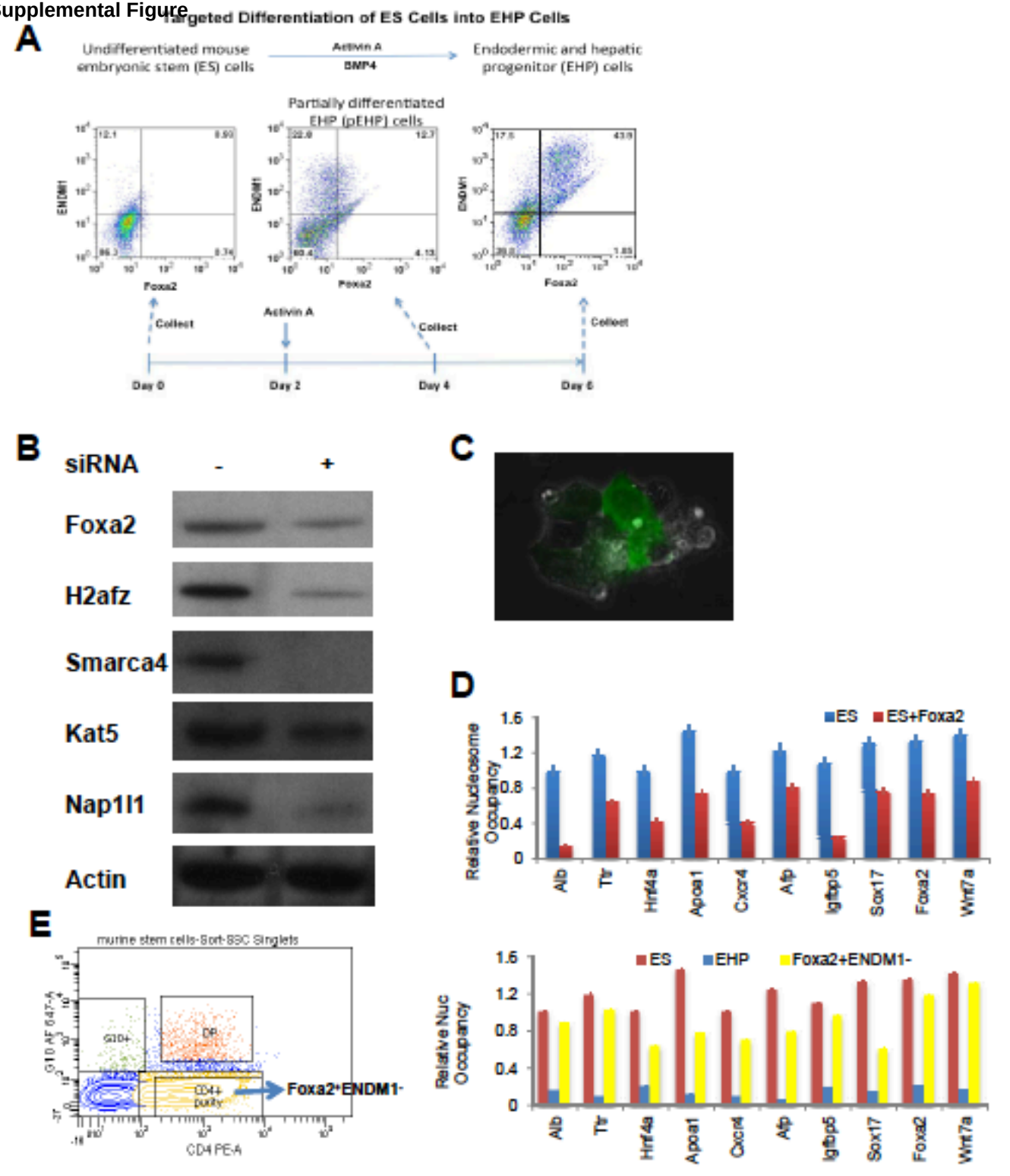

Supplemental Figure

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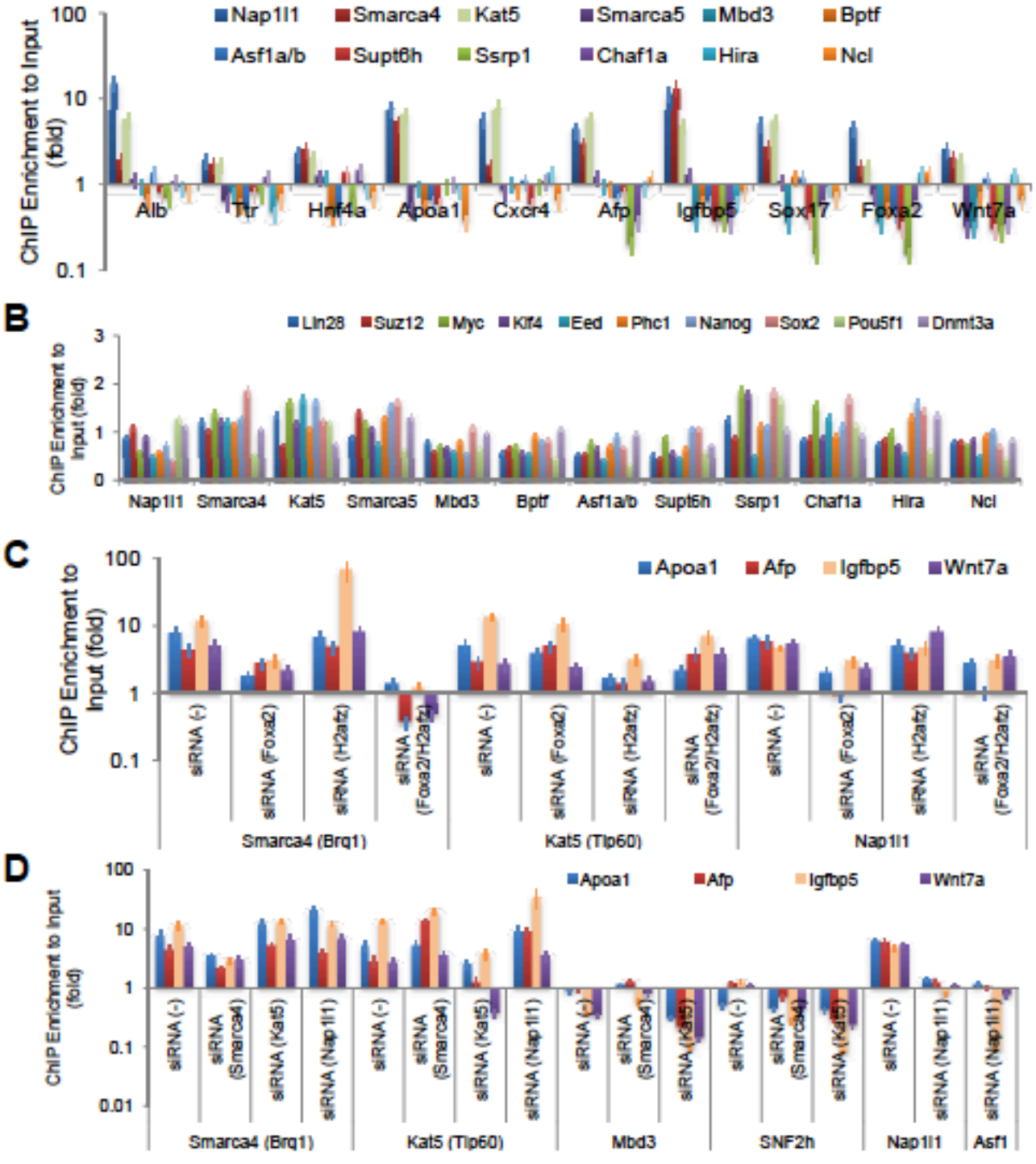

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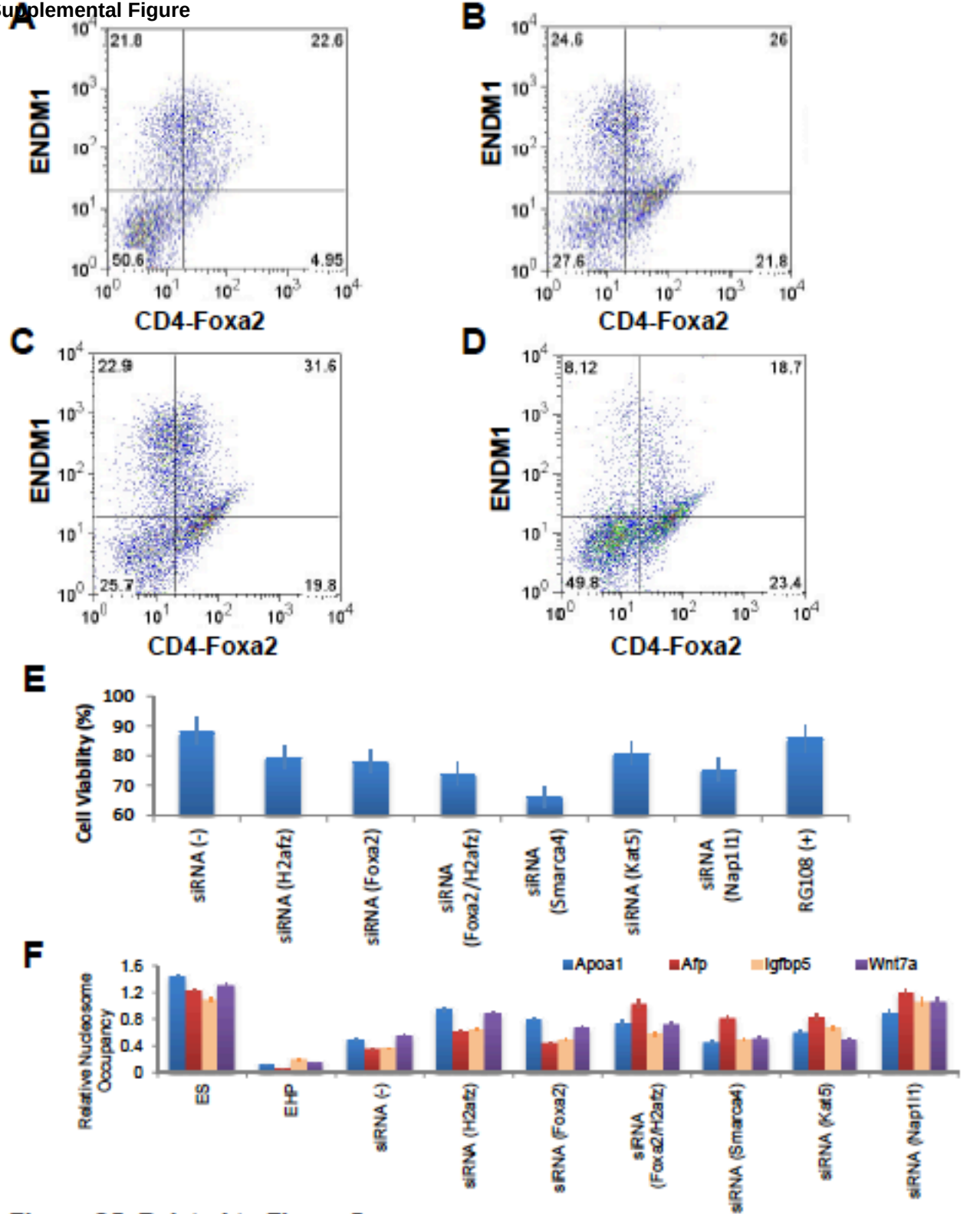

Figure S5. Related to Figure 5