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Supplemental Information

Nonreciprocal and Conditional Cooperativity Directs the Pioneer Activity of Pluripotency Transcription Factors

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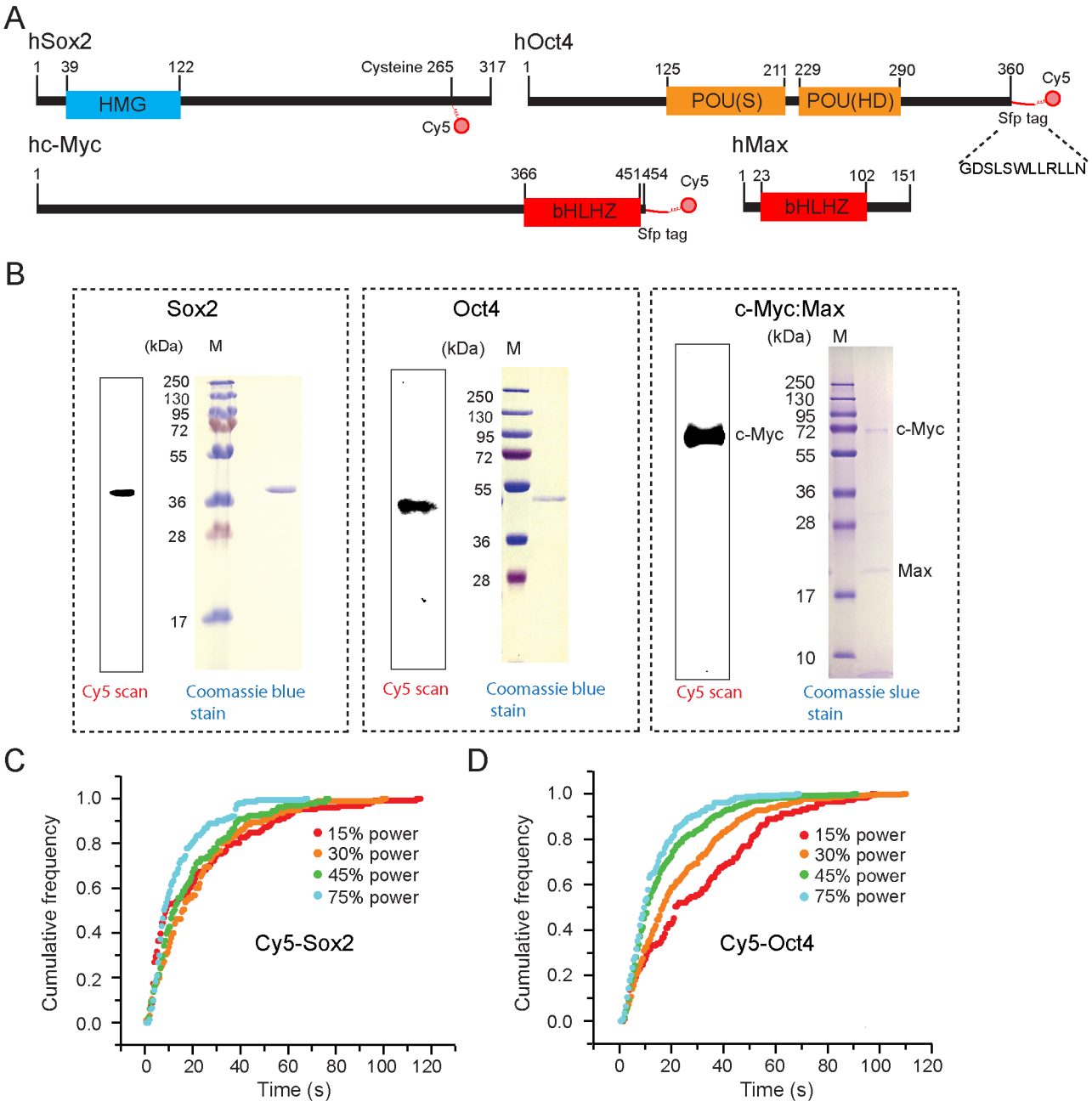

Figure S1. Site-Specific Labeling of Pluripotency TFs and Photobleaching Kinetics of Fluorescently Labeled TFs, Related to Figures 1 and 3

(A) Schematic of the full-length human Sox2, Oct4, c-Myc and Max proteins. Their DNA-binding domains are indicated.

(B) SDS-PAGE analysis of Cy5-labeled Sox2, Oct4, and c-Myc:Max heterodimer.

(C) Cumulative distributions of the observed Cy5-labeled Sox2 residence time on DNA measured at different levels of laser power.

(D) Same as (C) except for analyzing Cy5-labeled Oct4. In (C) and (D), the photobleaching rate constant (k_{bleach}) is assumed to be linearly dependent on the laser power at non-saturating conditions. As such,

k_{bleach} can be calculated by solving $k_{off,obs} = k_{bleach} + k_{off}$ at multiple laser powers, with $k_{off,obs}$ and k_{off} representing the observed TF dissociation rate constant and the true dissociation rate constant, respectively. At 30% power, the time constants for Cy5-Sox2 photobleaching and Cy5-Oct4 photobleaching are calculated to be 75 s and 42 s, respectively.

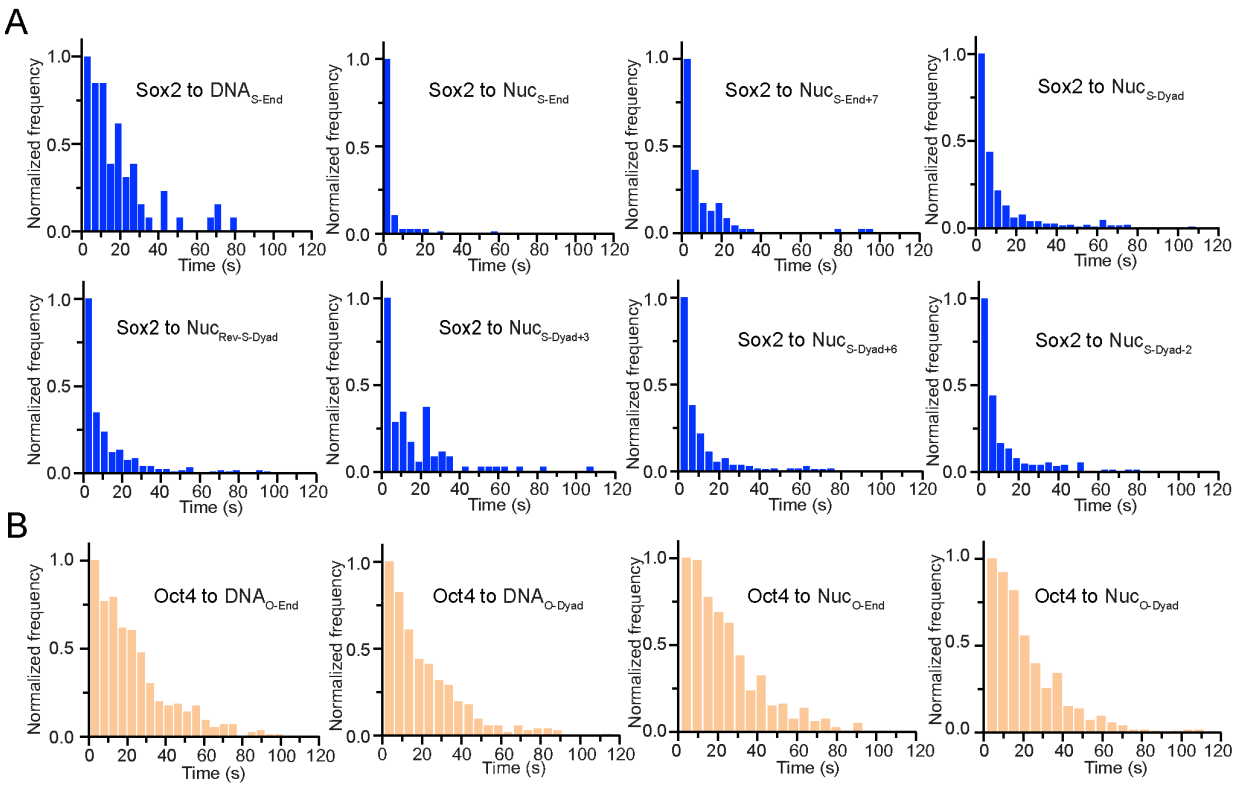

Figure S2. Histograms of TF residence time on different DNA and nucleosome substrates, Related to Figures 1, 2, and 3

(A) Histograms of Sox2 residence time.

(B) Histograms of Oct4 residence time.

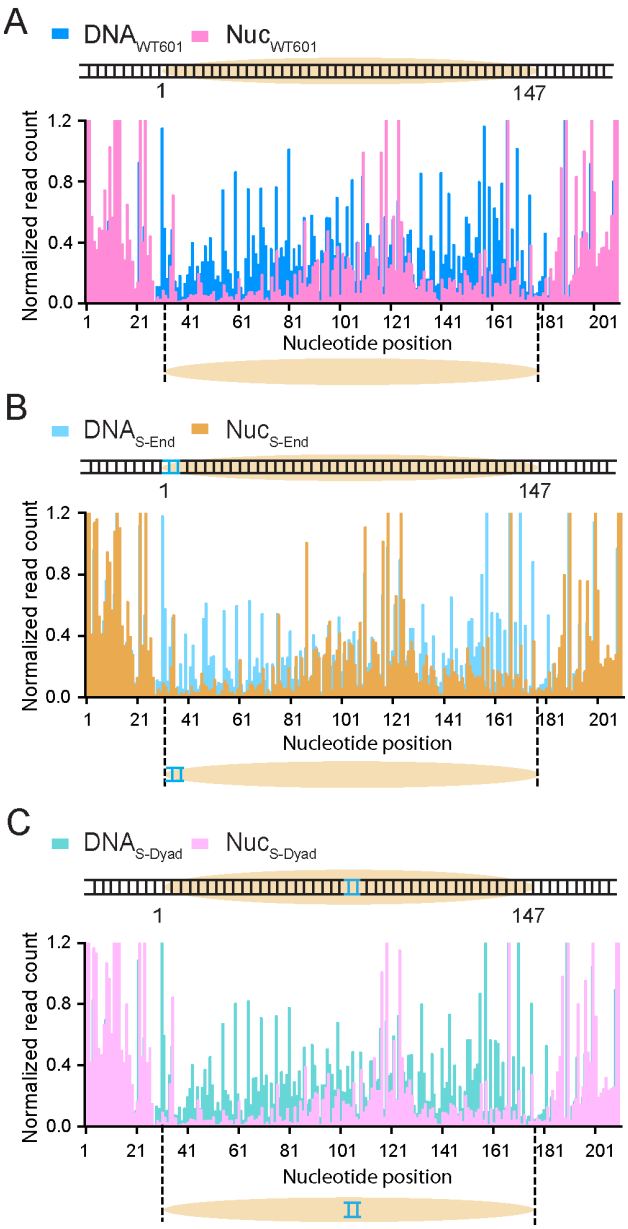

Figure S3. Evaluating Nucleosome Positioning Using DNase Footprinting, Related to Figures 1 and 2
(A) DNase I footprinting pattern for a DNA substrate containing a wildtype 601 NPS (DNA_{WT601}, blue) or a mononucleosome substrate reconstituted from the same DNA template (Nuc_{WT601}, pink). The DNase-protected region in the Nuc_{WT601} data reflects the position of the nucleosome.
(B) Same as (A), except with a DNA template containing a 7-bp-long Sox2 binding motif placed at the end of the 601 NPS (DNA_{S-End}, light blue; Nuc_{S-End}, brown).
(C) Same as (A), except with a DNA template containing a Sox2 binding motif placed at the dyad of the 601 NPS (DNA_{S-Dyad}, light green; Nuc_{S-Dyad}; light pink).
All three nucleosome constructs share a similar protection pattern, suggesting that nucleosome positioning is not perturbed by the engineered TF binding motifs.

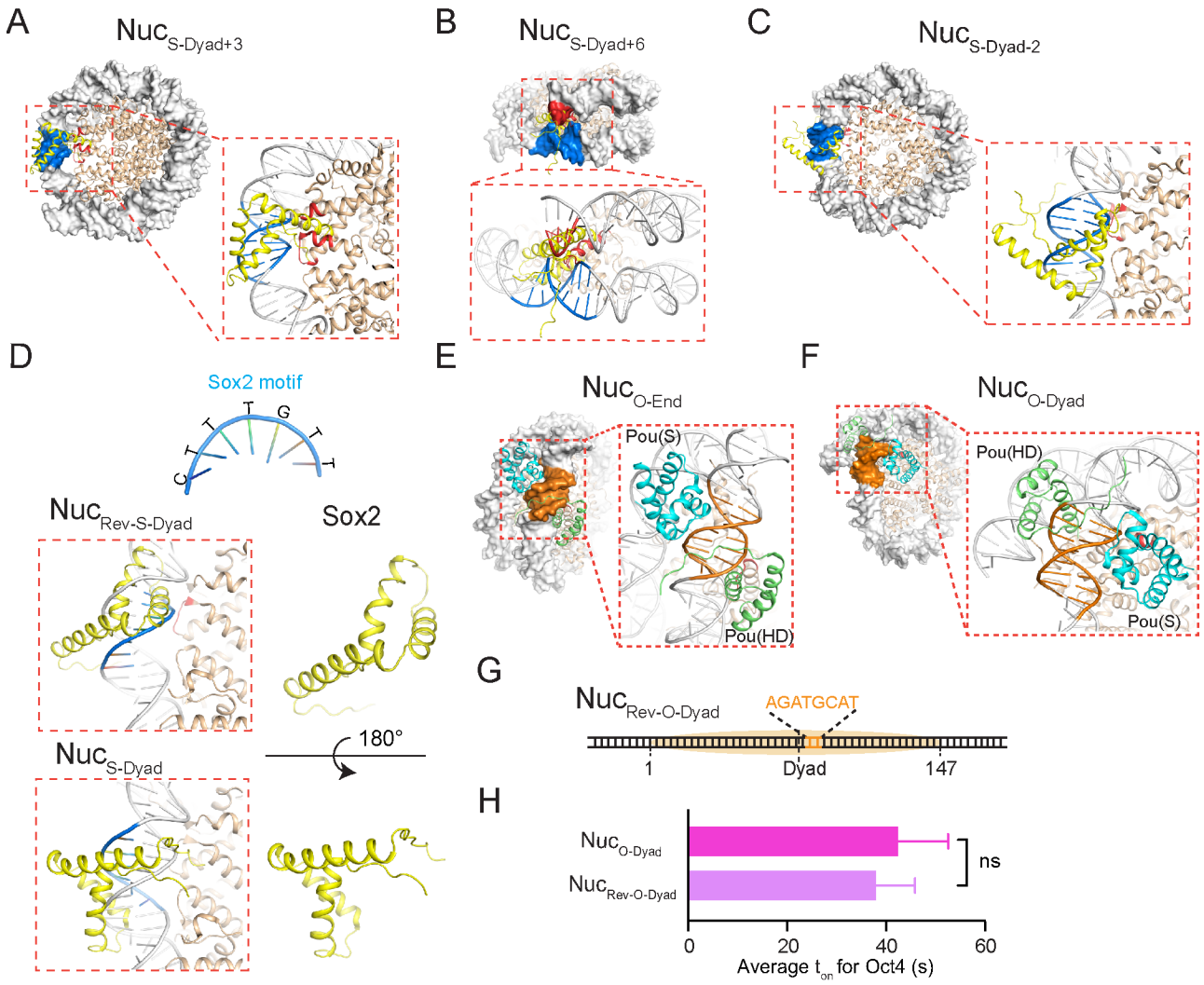

Figure S4. Additional Structural Modeling and Kinetic Analysis of Sox2 and Oct4 Binding to Nucleosome Substrates, Related to Figures 2 and 3

(A-C) The $Sox2_{HMG}$:DNA structure (PDB: 1GT0; yellow) superimposed on the 601 nucleosome structure (PDB: 3LZ0) aligned by the DNA motif (blue) located at the dyad+3 (A), dyad+6 (B), or dyad-2 (C) position. Steric clash between Sox2 and the nucleosome is highlighted in red.

(D) $Sox2_{HMG}$:DNA structure superimposed on a 601 nucleosome with a Sox2 binding motif CTTTGTT (blue) encoded in either the Watson or the Crick strand at the dyad position. In both cases the minor groove of the binding site faces outward, but Sox2 binds in mirrored directions.

(E and F) Superposition between the $Oct4_{POU}$:DNA structure (PDB: 1GT0) and the 601 nucleosome structure (PDB: 3LZ0) aligned by the DNA motif (orange) located at the end (E) or dyad (F) position. The Oct4 POU_{HD} and POU_S domains are shown in green and cyan, respectively.

(G) Diagram of the nucleosome substrate ($Nuc_{Rev\text{-}O\text{-}Dyad}$) containing a reverse Oct4 binding motif near the dyad.

(H) Average Oct4 residence times on $Nuc_{O\text{-}Dyad}$ and $Nuc_{Rev\text{-}O\text{-}Dyad}$.

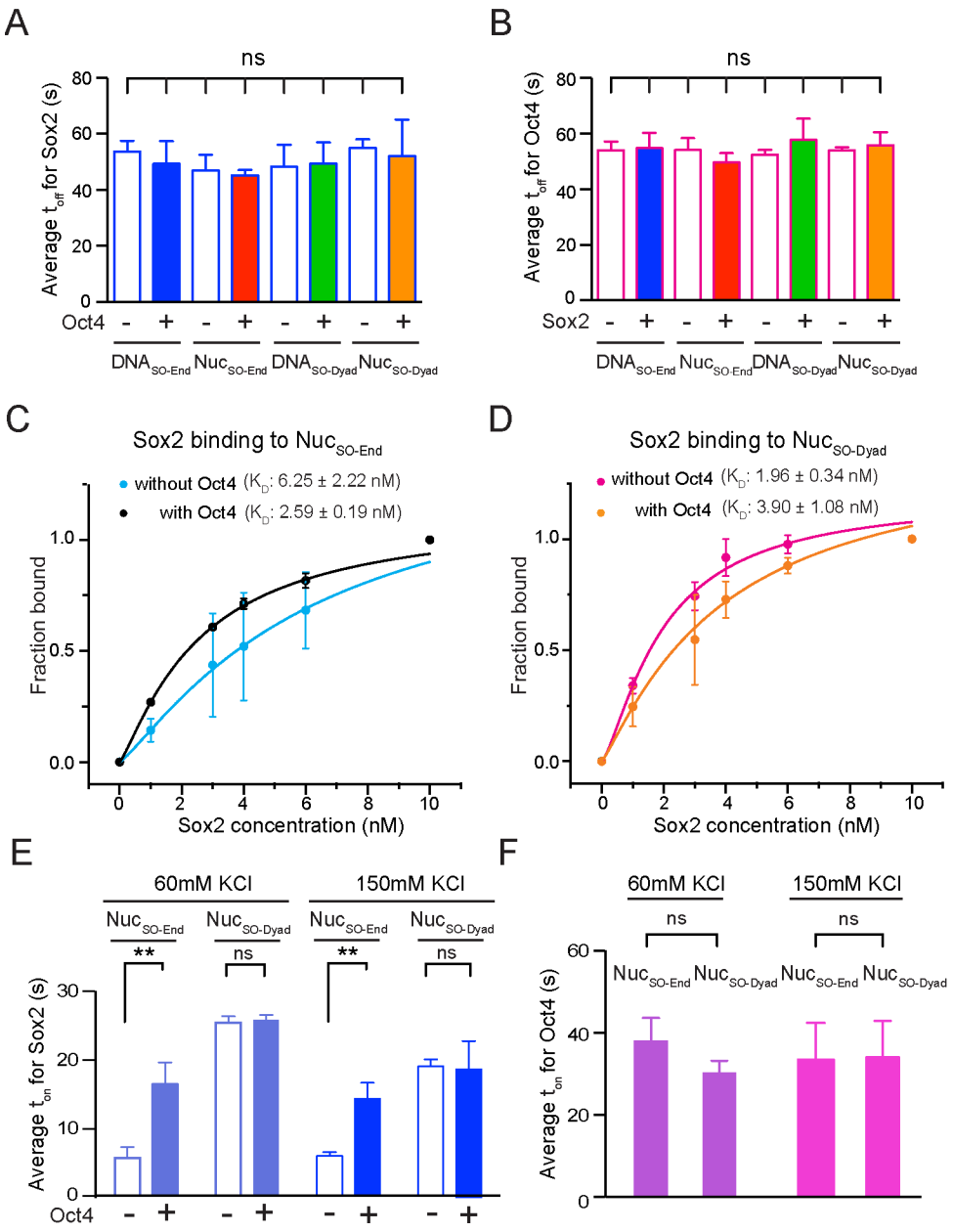

Figure S5. Additional Quantification of Sox2- and Oct4-Nucleosome Interactions, Related to Figures 4, 5, and 6

(A) Average waiting time (t_{off}) before Sox2 binding to a DNA or nucleosome substrate containing a composite Sox2:Oct4 motif, in the absence or presence of Oct4.

(B) Average waiting time before Oct4 binding to the same substrates as in (A) in the absence or presence of Sox2.

(C) Fraction of Nuc_{SO-End} substrates that were bound to Sox2 as a function of Sox2 concentration in the absence (blue circles) or presence (black circles) of 10 nM Oct4. K_D values were determined by fitting the data to a Hill function (blue and black curves). Hill coefficient n_{Hill} = 1.2 ± 0.1 without Oct4 and 1.2 ± 0.1 with Oct4.

(D) Same as (C), except for analyzing the Sox2:$Nuc_{SO\text{-}Dyad}$ interaction. n_{Hill} = 1.4 ± 0.2 without Oct4 and 1.2 ± 0.2 with Oct4.
(E) Average residence times (t_{on}) of Sox2 on $Nuc_{SO\text{-}End}$ and $Nuc_{SO\text{-}Dyad}$ in the absence or presence of Oct4 under different salt conditions.
(F) Average residence times of Oct4 on $Nuc_{SO\text{-}End}$ and $Nuc_{SO\text{-}Dyad}$ under different salt conditions.
Data are represented as mean ± SD.

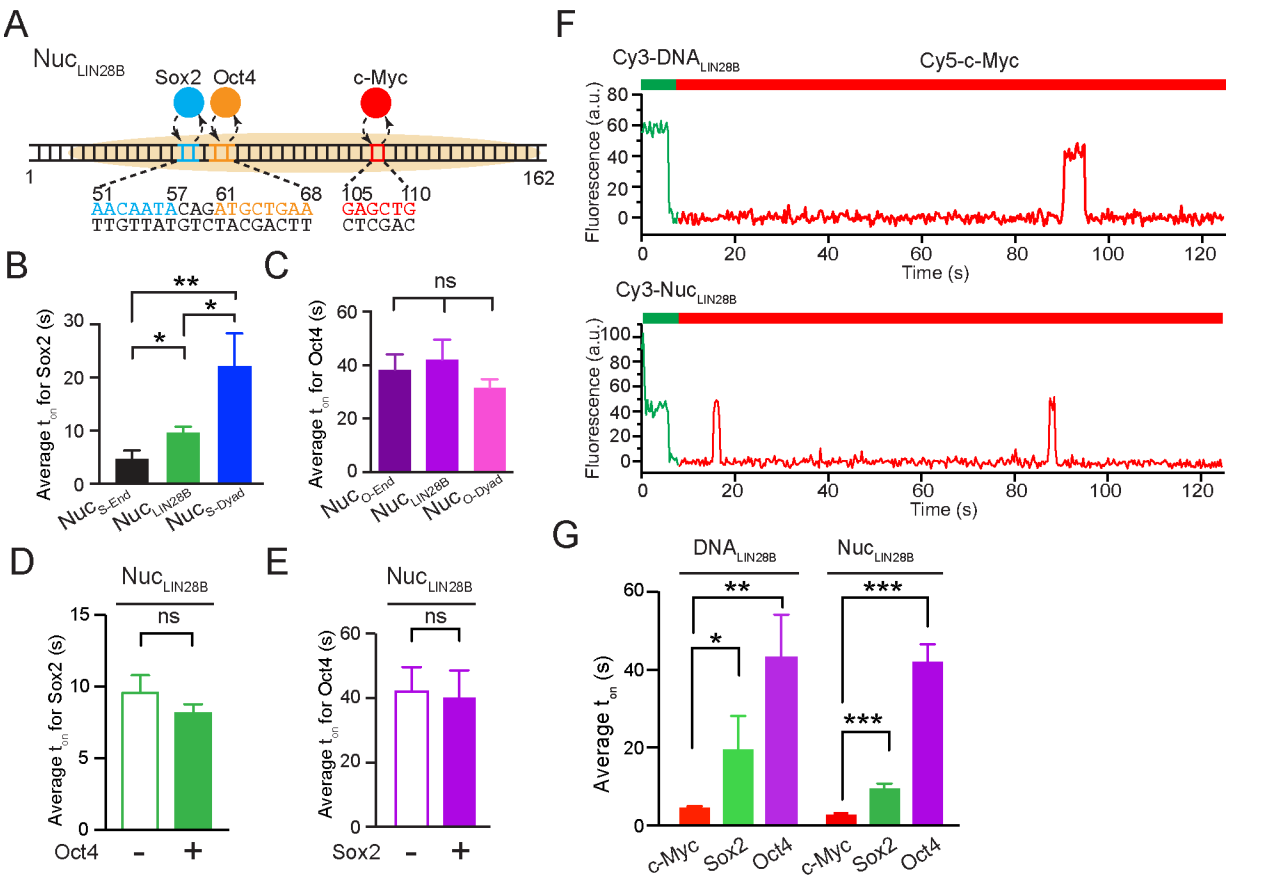

Figure S6. Pluripotency TFs Exhibit Distinct Binding Behaviors at a Native Genomic Locus, Related to Figures 4, 5, and 6

(A) Diagram of the *LIN28B* genomic locus. Positions of the predicted Sox2, Oct4, and c-Myc binding sites are indicated. The putative Sox2 and Oct4 sites are oppositely oriented.

(B) Average residence times of Sox2 on nucleosome substrates with differentially positioned Sox2 binding motifs.

(C) Average residence times of Oct4 on nucleosome substrates with differentially positioned Oct4 binding motifs.

(D) Average residence time of Sox2 on Nuc_{LIN28B} in the absence or presence of Oct4.

(E) Average residence time of Oct4 on Nuc_{LIN28B} in the absence or presence of Sox2.

(F) Representative fluorescence-time trajectories showing Cy5-labeled c-Myc:Max binding to Cy3-labeled DNA_{LIN28B} and Nuc_{LIN28B}.

(G) Comparison of the average residence time of c-Myc, Sox2, and Oct4 on DNA_{LIN28B} (left) and Nuc_{LIN28B} (right).

Data are represented as mean ± SD.

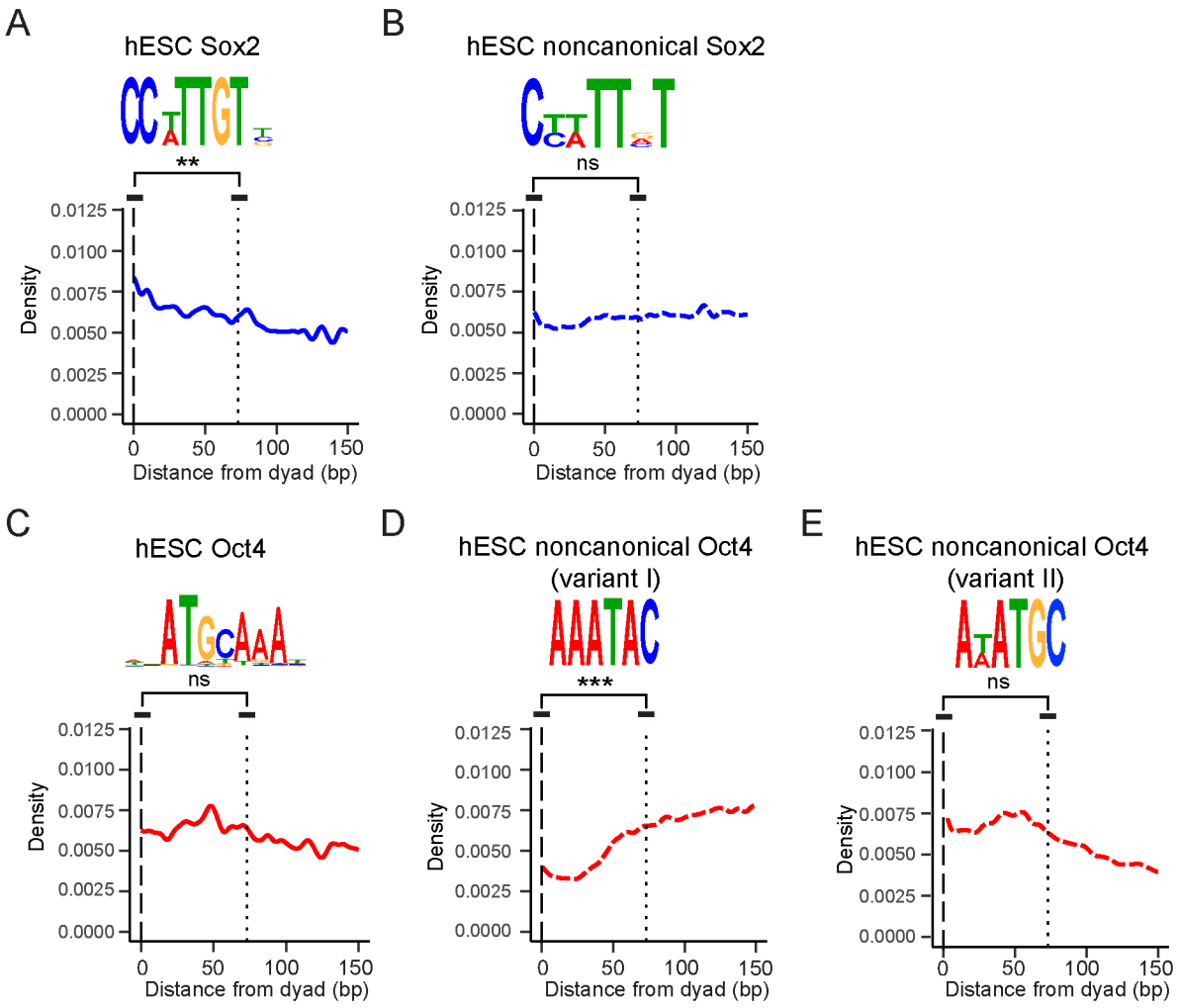

Figure S7. Analysis of Sox2 and Oct4 Binding Preference in Human Embryonic Stem Cells, Related to Figure 7

(A) Sequence logo of the canonical Sox2 binding motif (top) and smoothed (3-bp filter) distribution of the distance between a canonical Sox2 binding site and the nearest nucleosome dyad (bottom) in hESCs (N = 2,909). Position 0 (dashed line) corresponds to the dyad; the dotted line approximates the edges of the nucleosome. Displayed significance is from t-test conducted between a 13-bp window centered at the dyad and a 13-bp window inside the nucleosome edge (P = 0.0040).

(B) Same as (A), except for analyzing noncanonical Sox2 motifs (N = 9,264; P = 0.66).

(C) Same as (A), except for analyzing canonical Oct4 binding motifs (N = 5,455; P = 0.50).

(D) Same as (C), except for analyzing noncanonical Oct4 motifs (variant I) resembling one half of the canonical octameric Oct4 motif (N = 20,698; $P = 4.0 \times 10^{-8}$).

(E) Same as (C), except for analyzing noncanonical Oct4 motifs (variant II) resembling the other half of the canonical octameric Oct4 motif (N = 31,367; P = 0.51).

Table S1. Oligonucleotides used in this work. Related to STAR Methods

Oligonucleotides (TF binding motifs in bold)	Source
601 $DNA_{S\text{-}End}$	This paper
CTTTGTTATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{S\text{-}End+7}$	This paper
CTGGAGA**CTTTGTT**TGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{S\text{-}Dyad}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC G**CTTTGTT**CCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{S\text{-}Dyad+3}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGC**CTTTGTT**CGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{S\text{-}Dyad+6}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGCTGT**CTTTGTT**GTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{S\text{-}Dyad\text{-}2}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTA**C TTTGTT**TCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{Rev\text{-}S\text{-}Dyad}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC G**AACAAAG**CCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{O\text{-}End}$	This paper
CTGGAGA**ATGCATCT**GCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{O\text{-}Dyad}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGCTGTC**ATGCATCT**TTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{Rev\text{-}O\text{-}Dyad}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC GCGCTGTC**AGATGCAT**TTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{SO\text{-}End}$	This paper
CTTTGTTATGCATCTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTA CGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{Rev\text{-}SO\text{-}End}$	This paper
AACAAAGATGCATCTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTA CGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{SO+3\text{-}End}$	This paper
CTTTGTTTGG**ATGCATCT**GAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTA CGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{Rev\text{-}SO+3\text{-}End}$	This paper

AACAAAGTGG**ATGCATCT**GAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTA CGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATAC ATCCTGT	
601 $DNA_{SO\text{-}Dyad}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC G**CTTTGTTATGCATCT**TTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATA CATCCTGT	
601 $DNA_{SO+3\text{-}Dyad}$	This paper
CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTAC G**CTTTGTT**TGG**ATGCATCT**AACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCACATATATA CATCCTGT	
DNA_{LIN28B}	This paper
AGTGGTATTAACATATCCTCAGTGGTGAGTATTAACATGGAACTTACTCC**AACAATA**CAG**ATGCTGAA**T AAATGTAGTCTAAGTGAAGGAAGAAGGAAAGGTGG**GAGCTG**CCATCACTCAGAATTGTCCAGCAGGGAT TGTGCAAGCTTGTGAATAAAGACA	
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AmC6-601-R	IDT
/5AmMC6/TACGCGAATTCCAAGCGACACCGGCACT	
Biotin-lin28B-F	IDT
/5Biosg/AGTGGTATTAACATATCCTCAGTGGTG	
AmC6-lin28B-R	IDT
/5AmMC6/TGTCTTTATTCACAAGCTTGCACAA	
H2B T49C mutant-F	IDT
GATTGCGGCATCTCGTCCAAGGCCATG	
H2B T49C mutant-R	IDT
GGGGTGCACCTGCTTCAGCACCTTG	
Sox2-F	IDT
GGAATTCCATATGTACAACATGATGGAGACG	
Sox2-R	IDT
CCGCTCGAGTCACATGTGTGAGAGGGGC	
Oct4-F	IDT
GGAATTCCATATGGCGGGACACCTGGCTTCG	
Oct4-R	IDT
GCCGACGTCGACTCAGTTTGAATGCATGGGAG	
Oct4 with C-terminal Sfp tag-F	IDT
CTACTACGACTACTAAACTGAGTCGAGCACCACCACCACC	
Oct4 with C-terminal Sfp tag-R	IDT
CCATGATAGTGAGTCTCCGTTTGAATGCATGGGAGAGCCC	
c-Myc-F	IDT
GGAATTCCATATGCTGGATTTTTTTCGGGTAG	
c-Myc-R	IDT
CCGCTCGAGTTACGCACAAGAGTTCCGTAG	
c-Myc with C-terminal Sfp tag-F	IDT
CTATCATGGCTACTACGACTACTAAACTAACTCGAGCACCACCACCACCAC	
c-Myc with C-terminal Sfp tag-R	IDT
TCGTAGTAGCCATGATAGTGAGTCTCCCGCACAAGAGTTCCGTAGCTGTTC	
Max-F	IDT
GGAATTCCATATGAGCGATAACGATGACATCGAGG	
Max-R	IDT
CCGCTCGAGTTAGCTGGCCTCCATCCGGAG	