Transcription factor activity and nucleosome organisation in mitosis

Festuccia, N., Owens, N., et al.

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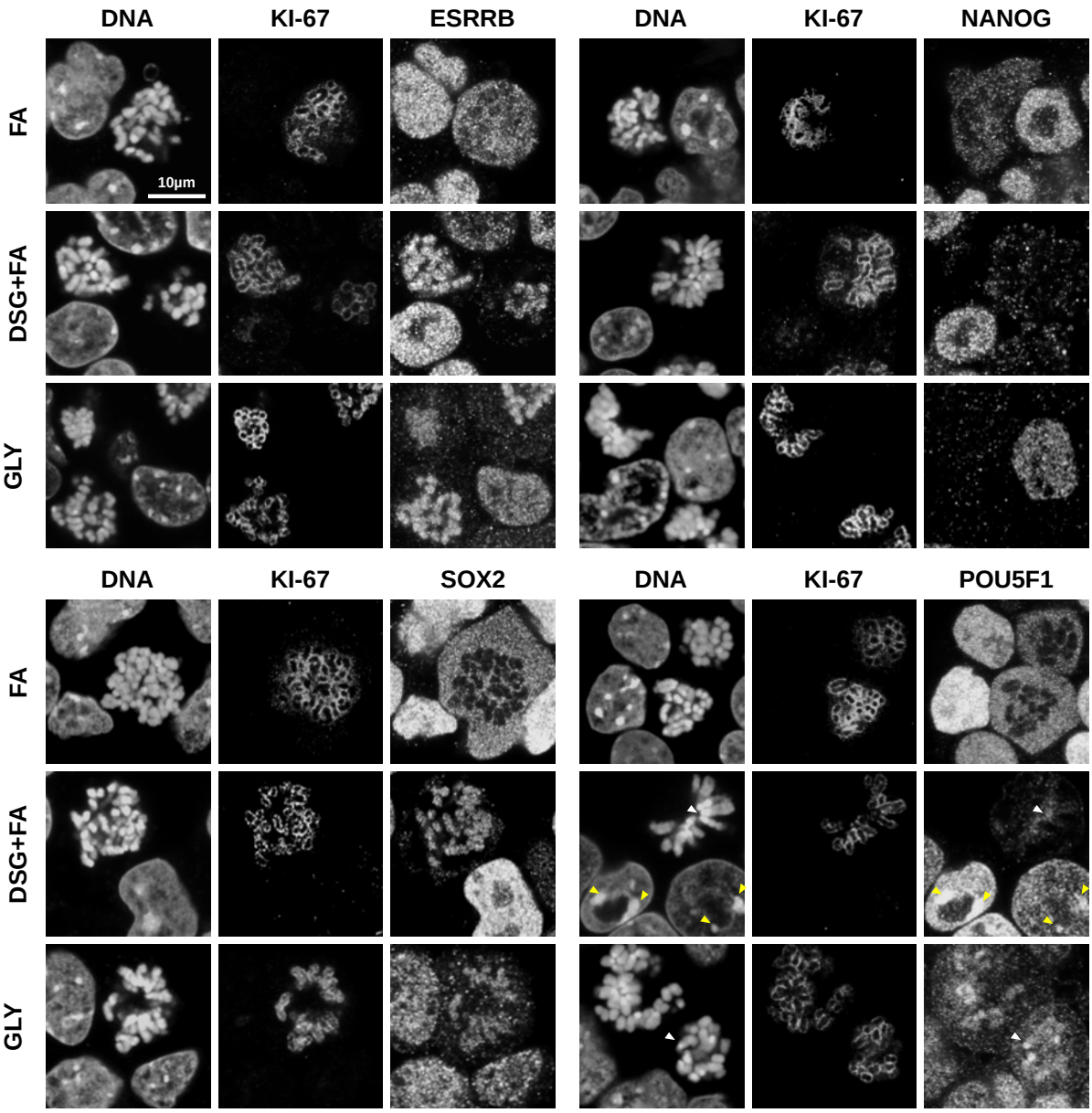
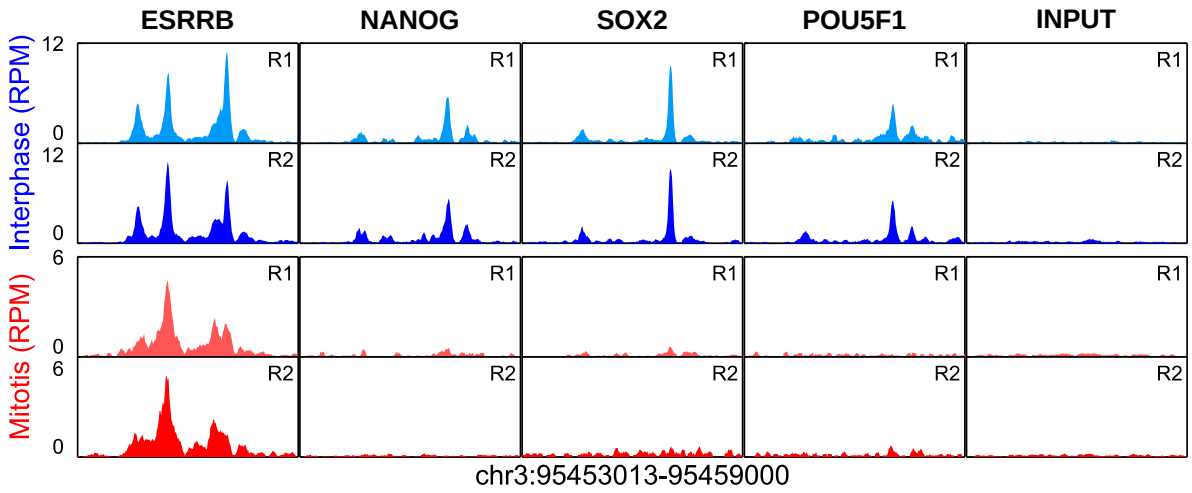
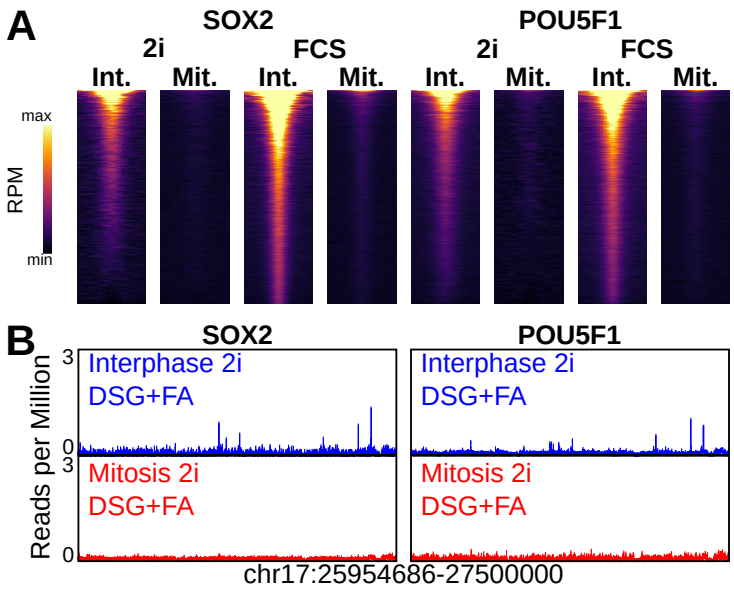


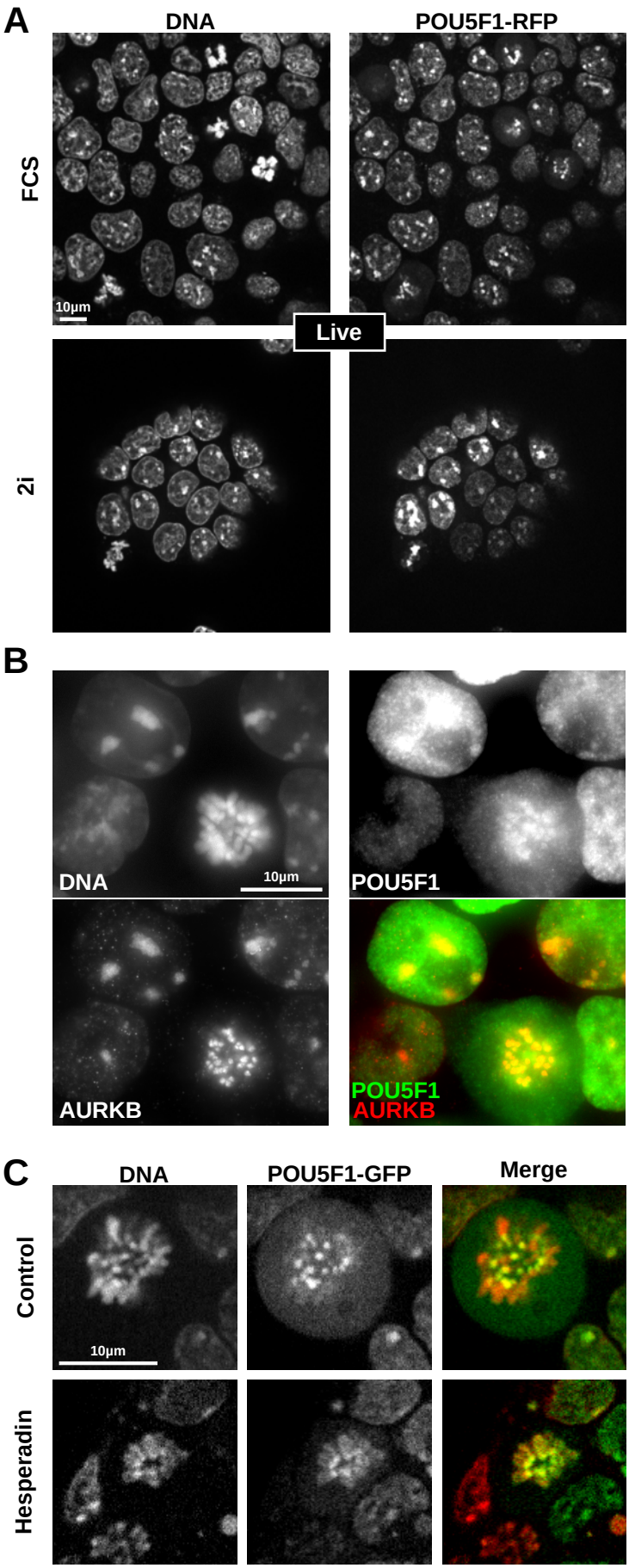
Supplemental Figure S1: Comparative analyses of different fixations. Immunofluorescence for ESRRB (top left), NANOG (top right), SOX2 (bottom left) and POU5F1 (bottom right), after fixation with either FA, DSG+FA, or glyoxal (GLY). DNA was counterstained with DAPI. The mitotic chromosome periphery is identified by KI-67 staining. These images correspond to the single channels of the merged images shown in Figs. 1 and 2. In the POU5F1 staining, the arrowheads indicate peri-centric heterochromatin foci (PCH) in interphase (yellow) and centromeres in mitosis (white). Note that SOX2 immunofluorescence required a FA post-fixation after Glyoxal.



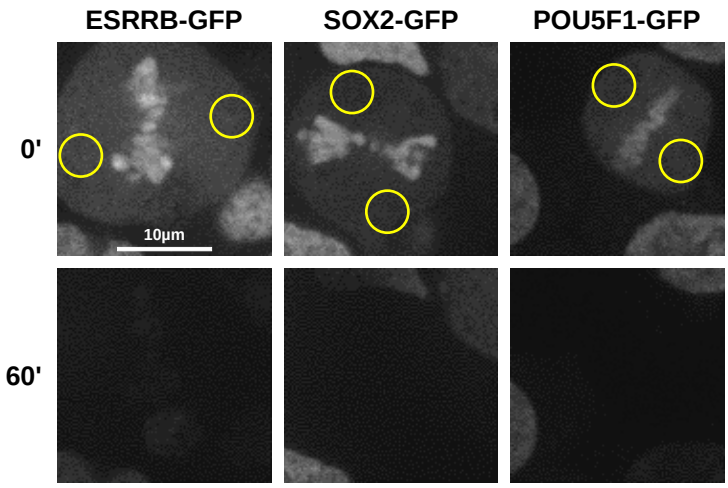
Supplemental Figure S2: Closer visualisation of a 6kb-long genomic region. Binding profiles of ESRRB, NANOG, SOX2 and POU5F1, along signal levels detected in INPUT samples (Reads per Million; RPM), in interphase (blue) or mitosis (red), obtained after fixation with DSG+FA. Two replicates are shown for each.



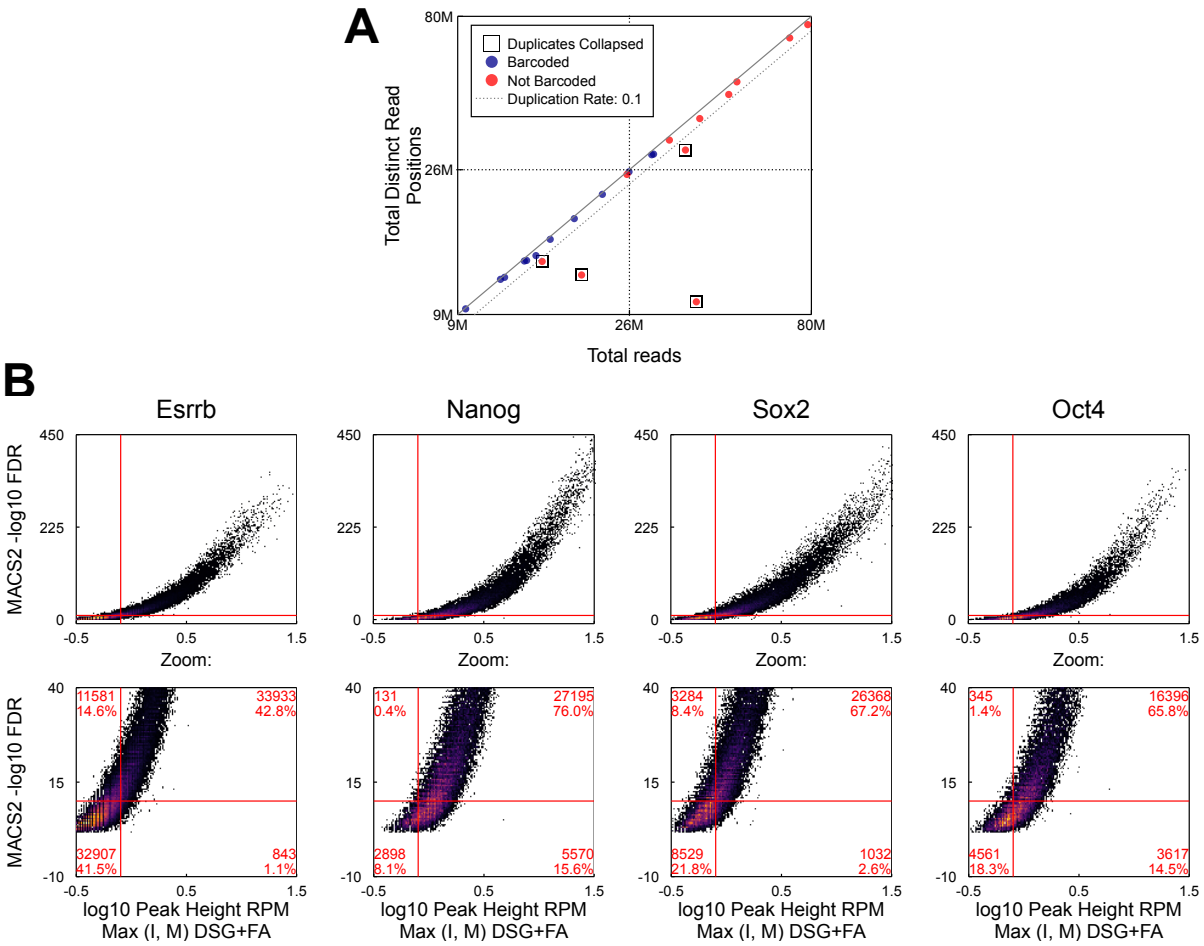
Supplemental Figure S3: POU5F1 and SOX2 binding in 2i-treated ES cells. (A) Heatmaps of ChIP-seq signal in interphase and mitosis for SOX2 and POU5F1, in cells cultured in the presence of 2 inhibitors (2i) of MEK and GSK3b or not (FCS). Binding regions are the union of peaks identified in both conditions. The heatmap (0 to 1 Reads per Million) is presented as in Fig. 4A. **(B)** Representative binding profile for SOX2 (left) or POU5F1 (right) in interphase (blue) or mitosis (red), obtained after fixation with DSG+FA in 2i treated cells; vertical scale Reads per Million. The region corresponds exactly to that shown in Figs. 1 and 2.



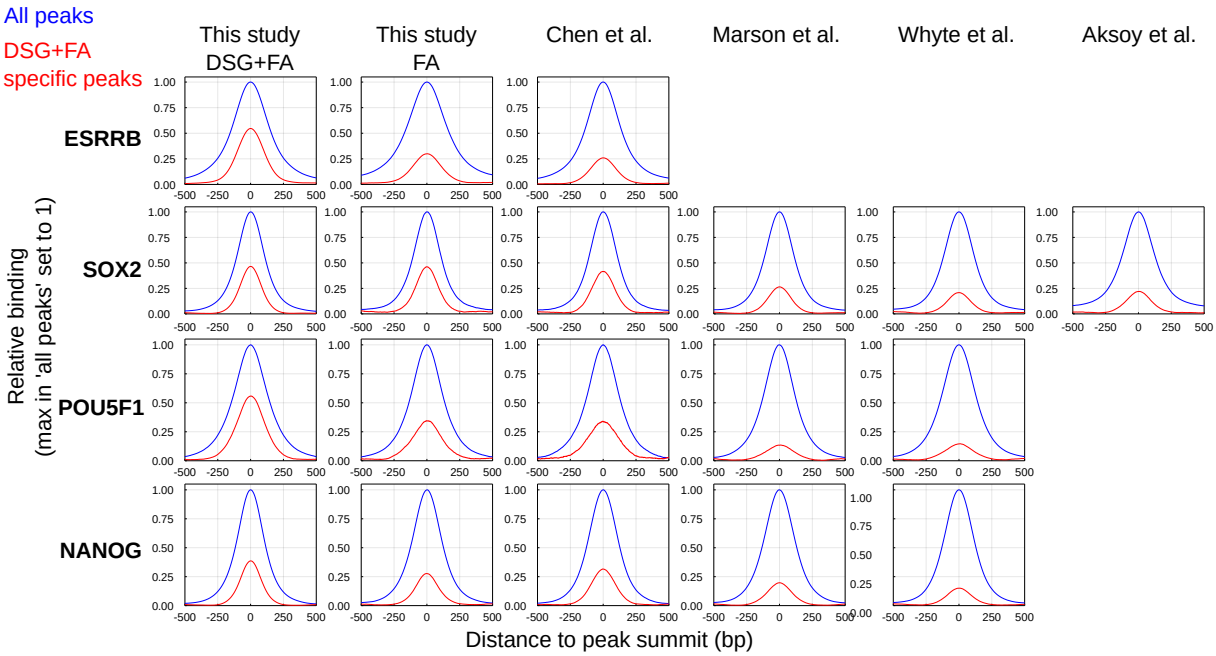
Supplemental Figure S4: Extended analysis of POU5F1 localisation. (A) Localisation of POU5F1-RFP fusion proteins expressed from one of the two endogenous *Pou5f1* alleles in live cells cultured in regular conditions (FCS/LIF medium; top) or in the presence of 2 inhibitors (2i; bottom) of MEK and GSK3b. DNA is visualised by Hoechst 33342 (red). **(B)** POU5F1 (green in the merge) and AURKB (red in the merge) immunofluorescence after fixation with DSG+FA. Note the large overlap at PCH and at centromeres in interphase and in mitosis. **(C)** Localisation of POU5F1-GFP fusion proteins in live cells cultured in the presence (bottom) or absence (top) of the AURKB inhibitor Hesperadin. DNA is visualised by Hoechst 33342 (red). Note this image corresponds exactly to that shown in Fig. 3A.



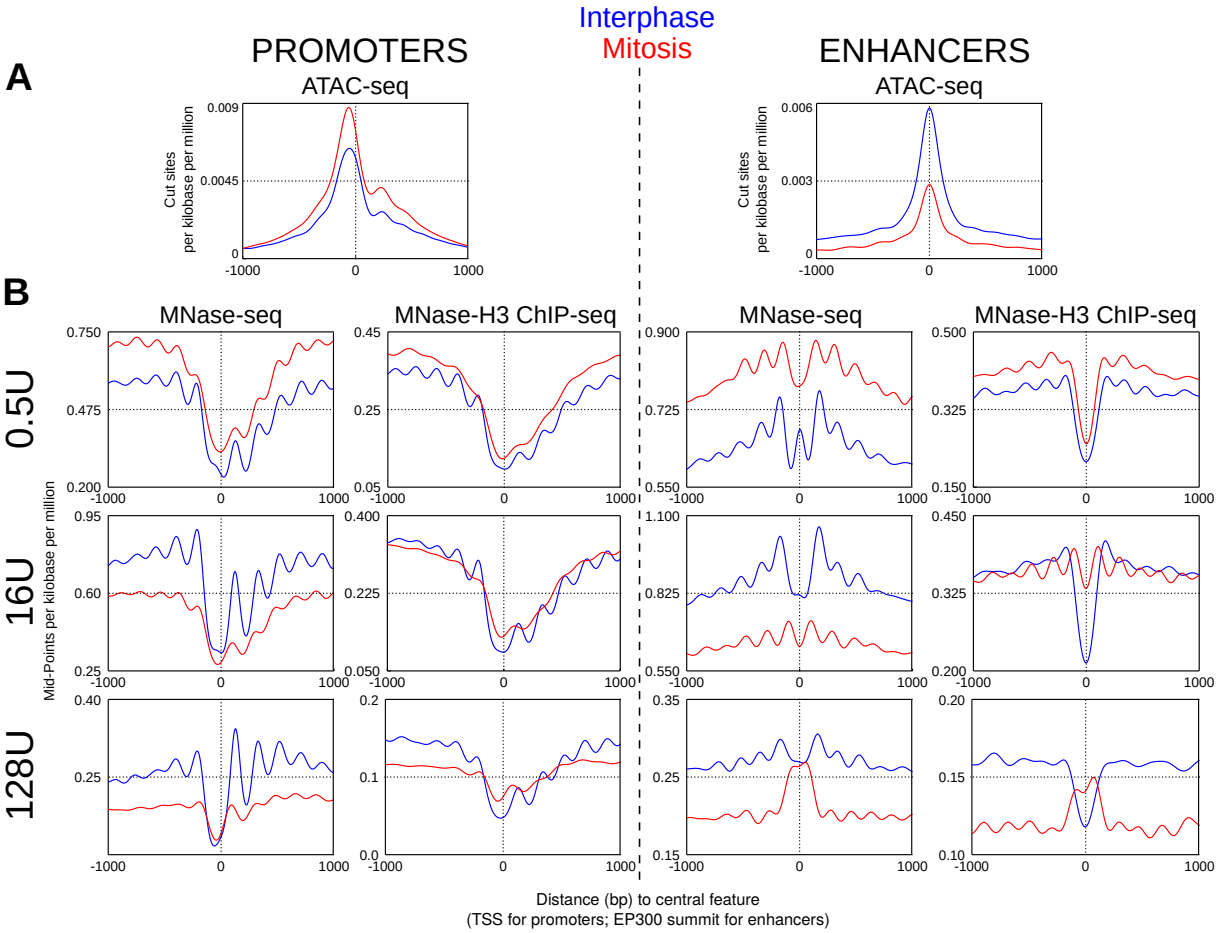
Supplemental Figure S5: Example of FLIP imaging. Representative examples of ESRRB-GFP, SOX2-GFP and POU5F1-GFP signal on mitotic chromosomes before and after 60 seconds of continuous bleaching of freely diffusing molecules outside the chromatids. The bleached areas are shown with yellow circles.



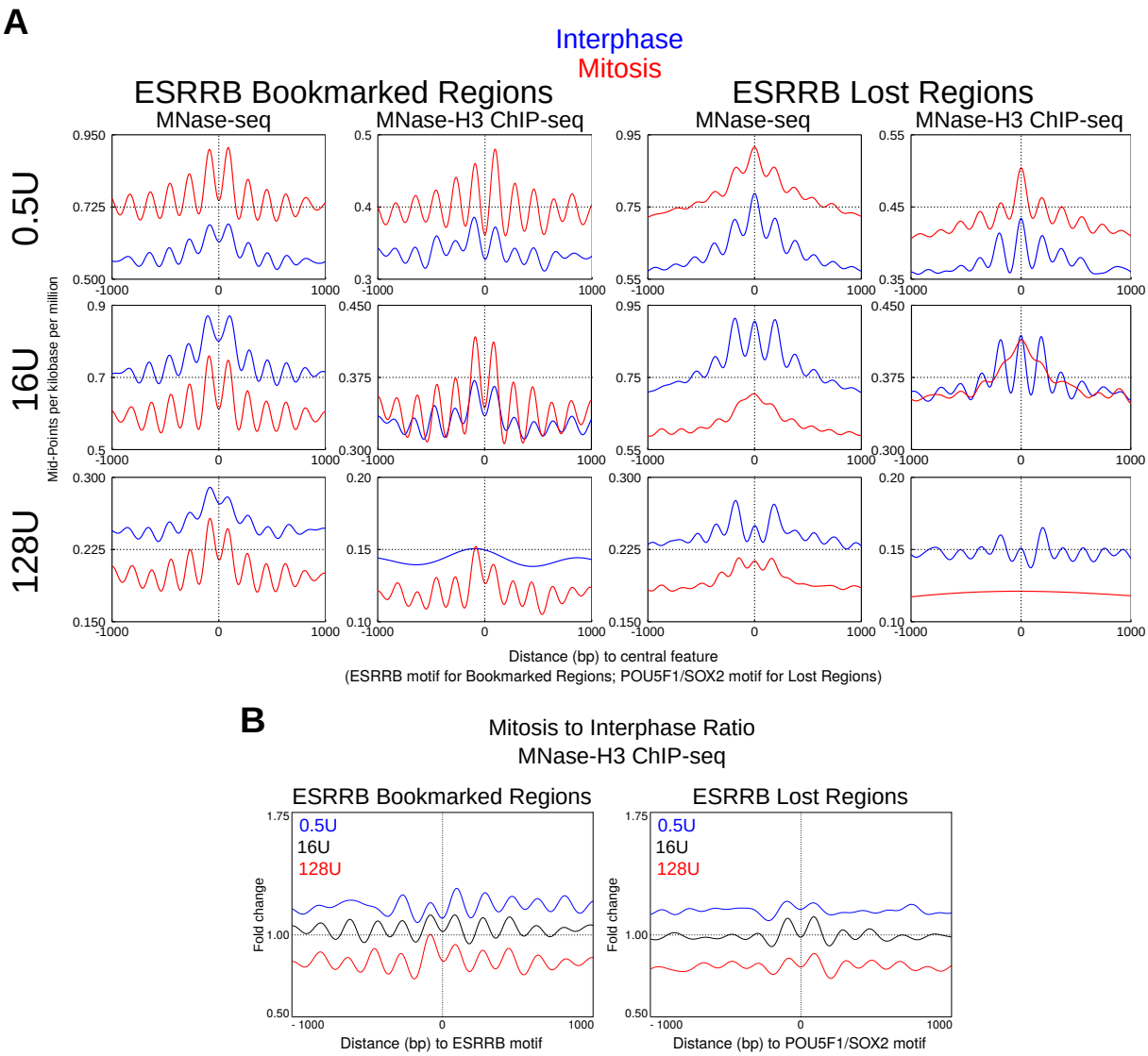
Supplemental Figure S6: Overview of TF binding sites identification. (A) For all libraries that were not constructed with random barcodes (red dots in the plot; see Supplemental Table S1 and Supplemental Methods) we collapsed duplicated reads into one only when the duplication rate in the library was above 10% (squared dots in the plot). See Supplemental Methods for details. **(B)** For all TF ChIP-seq datasets, we only considered MACS2 peaks with a height and FDR above the indicated thresholds (see Supplemental Methods for details).



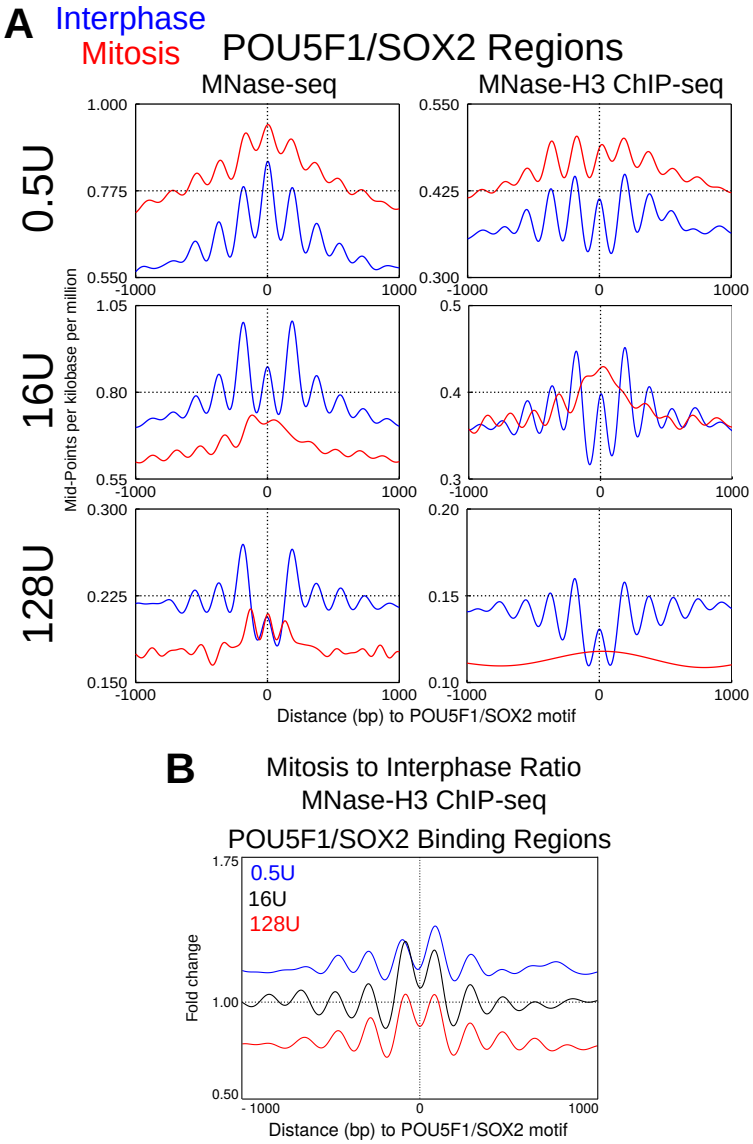
Supplemental Figure S7: Analysis of peaks specifically detected in DSG+FA. Average binding profile in interphase of ESRRB, SOX2, POU5F1 and NANOG, in this study and in public datasets. Blue line depicts all binding regions identified in this study, red depicts regions detected in DSG+FA exclusively, i.e. regions with no significant peak in any of the indicated FA datasets.



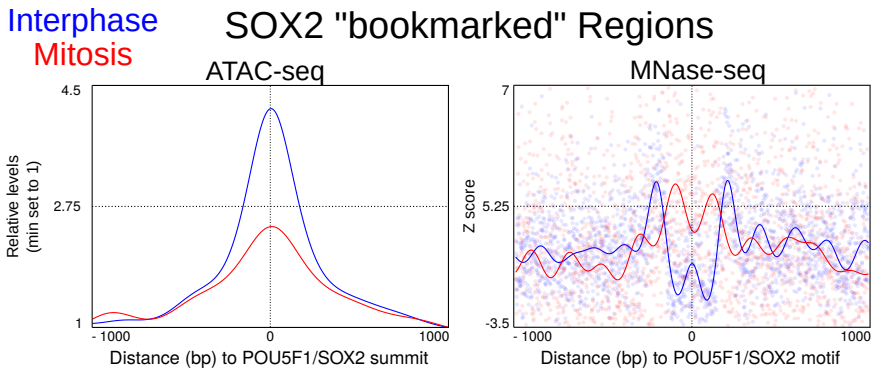
Supplemental Figure S8: Full ATAC-seq, MNase-seq and MNase-H3 ChIP-seq datasets at promoters and enhancers. (A) Chromatin accessibility at promoters (left) and enhancers (right) by ATAC-seq in interphase (blue) and mitosis (red). The Y axis shows the number of cut sites per base per million. To focus on differences in local enrichment over background, we use a minimum accessibility normalisation in Fig. 5A, D (see Supplemental Methods for details). **(B)** Nucleosome positioning at promoters (left) and enhancers (right), established by increased digestion with MNase, as indicated on the left (0.5U; 16U; 128U), in interphase (blue) and mitosis (red). The Y axis shows the number of midpoints of nucleosome-sized fragments (140-200bp) per base, per kilobase and per million fragments. Lines are Gaussian process regression of midpoint data. To generate the corresponding Fig. 5B, E, we used a z-score transformation (see Supplemental Methods for details) to compare differences in positioning rather than in magnitude. The X axis shows the genomic position centred on TSS for promoters and EP300 summits for enhancers.



Supplemental Figure S9: Full MNase and MNase-H3 ChIP-seq datasets at ESRRB binding regions. (A) Nucleosome positioning at bookmarked (left) and not-bookmarked (Lost; right) ESRRB binding regions, established by increased digestion with MNase, as indicated on the left (0.5U; 16U; 128U), in interphase (blue) and mitosis (red). The Y axis shows the number of midpoints of nucleosome-sized fragments (140-200bp) per base, per kilobase and per million fragments. Lines are Gaussian process regression of midpoint data. The X axis shows the genomic position centred on ESRRB motifs for bookmarked regions and on POU5F1/SOX2 motifs for lost regions. **(B)** Mitosis over interphase ratio of MNase-H3 ChIP-seq signal for nucleosomal fragments for MNase digestions with 0.5U (blue), 16U (black) and 128U (red) of enzyme, at bookmarked (left) and lost (right) regions.



Supplemental Figure S10: Full MNase and MNase-H3 ChIP-seq datasets at POU5F1/SOX2 binding regions. (A) Nucleosome positioning at POU5F1/SOX2 binding regions, established by increased digestion with MNase, as indicated on the left (0.5U; 16U; 128U), in interphase (blue) and mitosis (red). The Y axis shows the number of midpoints of nucleosome-sized fragments (140-200bp) per base, per kilobase and per million fragments. Lines are Gaussian process regression of midpoint data. The X axis shows the genomic position centred on POU5F1/SOX2 motifs **(B)** Mitosis over interphase ratio of MNase-H3 ChIP-seq signal for nucleosomal fragments for MNase digestions with 0.5U (blue), 16U (black) and 128U (red) of enzyme, at bookmarked (left) and lost (right) regions.



Supplemental Figure S11: Chromatin analysis of pseudo-bookmarked SOX2 binding regions. Left: Accessibility measured by cut sites of 0-100 bp ATAC-seq fragments at SOX2 putative bookmarked sites in interphase and mitosis, centred on SOX2 peak summits. Right: Nucleosome positioning measured by MNase-seq nucleosomal size fragments (140-200 bp) after digestion with 16U at SOX2 putative bookmarked sites centred on POU5F1/SOX2 motif. Vertical scale gives z-score.