

## SUPPLEMENTAL FIGURES TITLES AND LEGENDS

### **FIGURE S1 (related to Figure 1): H2B radius of confinement and average displacement define chromatin mobility populations**

A-B: Mean-Squared Displacement (MSD) in function of the delay (in seconds) (A) and aspect of the motion tracks (B) for unfiltered histone H2B motion tracks generated by FastSMT in one nucleus (see STAR methods).

C-D : Logarithmic frequency distribution of the diffusion coefficients of histone H2B (C) and dCas9 expressed without a guide RNA (noted DCAS9X) (D). Red arrow: low diffusing histone H2B molecules; Green arrow: high diffusing molecules; Empty red arrow: Negligible low-diffusing DCAS9X molecules.

E-H: Mean-Squared Displacement (MSD) in function of the delay (s), and histone H2B motion tracks generated by FastSMT in one nucleus, after different filtration methods (see STAR methods). E-F: selection of confined tracks with low  $\alpha$ -coefficients only (see STAR Methods); G-H: implementation of an additional selection criteria: a maximal jump threshold, indicating the efficient selection of confined motion tracks.

I: Scatter plot representing the radius of confinement vs. track duration for a subset of histone H2B motion tracks (n=19,668).

J: Scatter plot representing the average displacement vs. track duration for a subset of histone H2B motion tracks (n=5,312).

K Scatter plot of radius of confinement and average displacement assigned to n=104,602 single particle motions of histone H2B. The black line indicates a general positive correlation, and the black arrows indicate visible deviations from this line.

L: Schematic examples of motion tracks with similar/different average displacements (ave. displ). and similar/different radius of confinement (Radius Conf.).

M: Contours of the Voronoi diagrams indicating the centers of different H2B mobility groups (arrows) : A: low mobility; B and C: intermediate mobility (red arrows indicate the 3 clusters grouped to define population C; D: high mobility.

N-Q: Scatter density plots of the radius of confinement vs. average displacement for Panc-1 (M), BJ fibroblasts (N), MEFs (O) and iPS cells (P). vL: vMLC, L: LMC, I: IMC, H: HMC, vH: vHMC. Red arrows indicate the absence of vLMC and LMC in iPS cells.

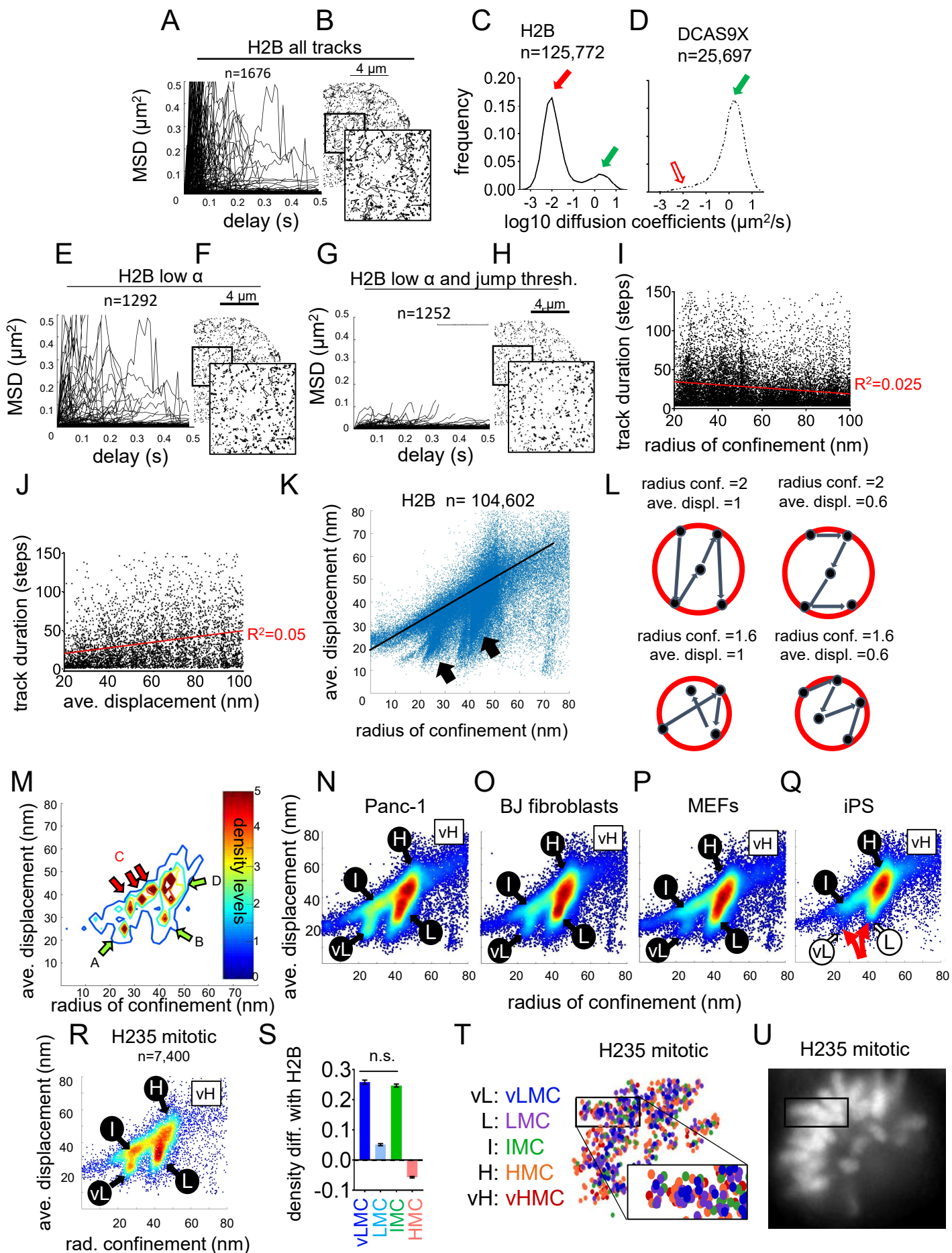
Q: Scatter density plots of the radius of confinement vs. average displacement for mitotic H2.35 cells. vL: vMLC, L: LMC, I: IMC, H: HMC, vH: vHMC.

R: Relative density levels of H2B in mitotic cells compared to histone H2B in vLMC, LMC, IMC, HMC and vHMC. For graphic simplification, we indicate only significant differences (n.s.), with  $p > 0.05$ , as determined by one-way ANOVA (see Table S1).

R: Subnuclear localization of the five mobility populations of histone H2B

S: Mitotic cell expressing H2B-Halo

# Lerner et al. Supplemental Figure 1



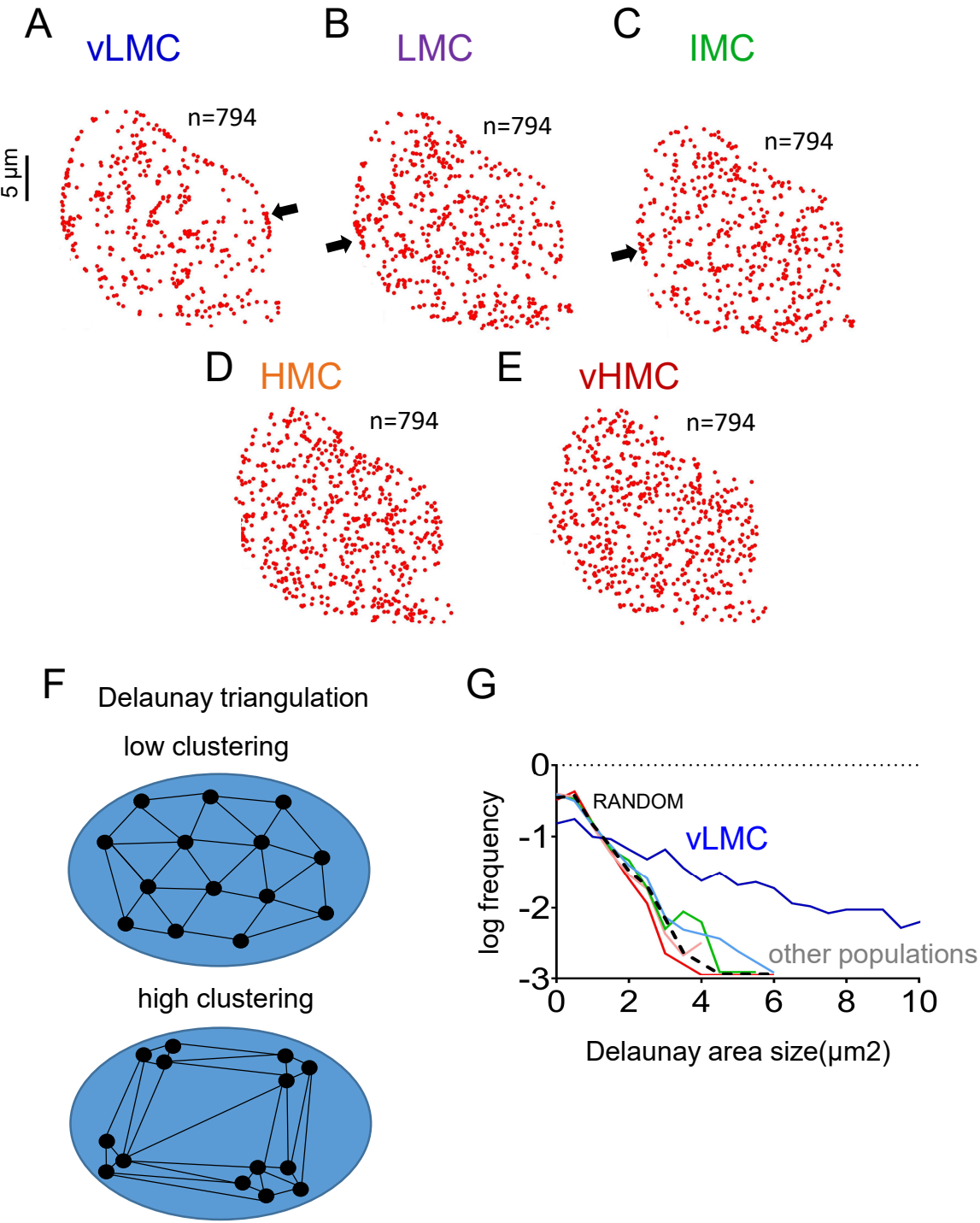
**FIGURE S2 (related to Figure 1): Subnuclear localization of chromatin mobility groups**

A-E : Subnuclear localization of vLMC (A), LMC (B), IMC (C), HMC (D) and vHMC (E) mobility H2B motion tracks (n=794). Each individual point corresponds to the average coordinates of each motion track. Black arrow indicates the presence of vLMC, LMC and IMC at the nuclear periphery.

F: Explanatory diagram for Delaunay triangulation. Spatial clustering leads to differences in the distribution of Delaunay area sizes.

G: Logarithmic frequency distribution of Delaunay area sizes for the different chromatin mobility populations.

Lerner et al. Supplemental Figure 2



**FIGURE S3 (Related to Figure 3): HP1 isoforms show degrees of variability in their mobility patterns**

A-C: Scatter density plots of radius of confinement and average displacements for H2B (A) HP1 $\beta$  (B) and HP1 $\gamma$  (C). vL: vMLC, L: LMC, I: IMC, H: HMC, vH: vHMC.

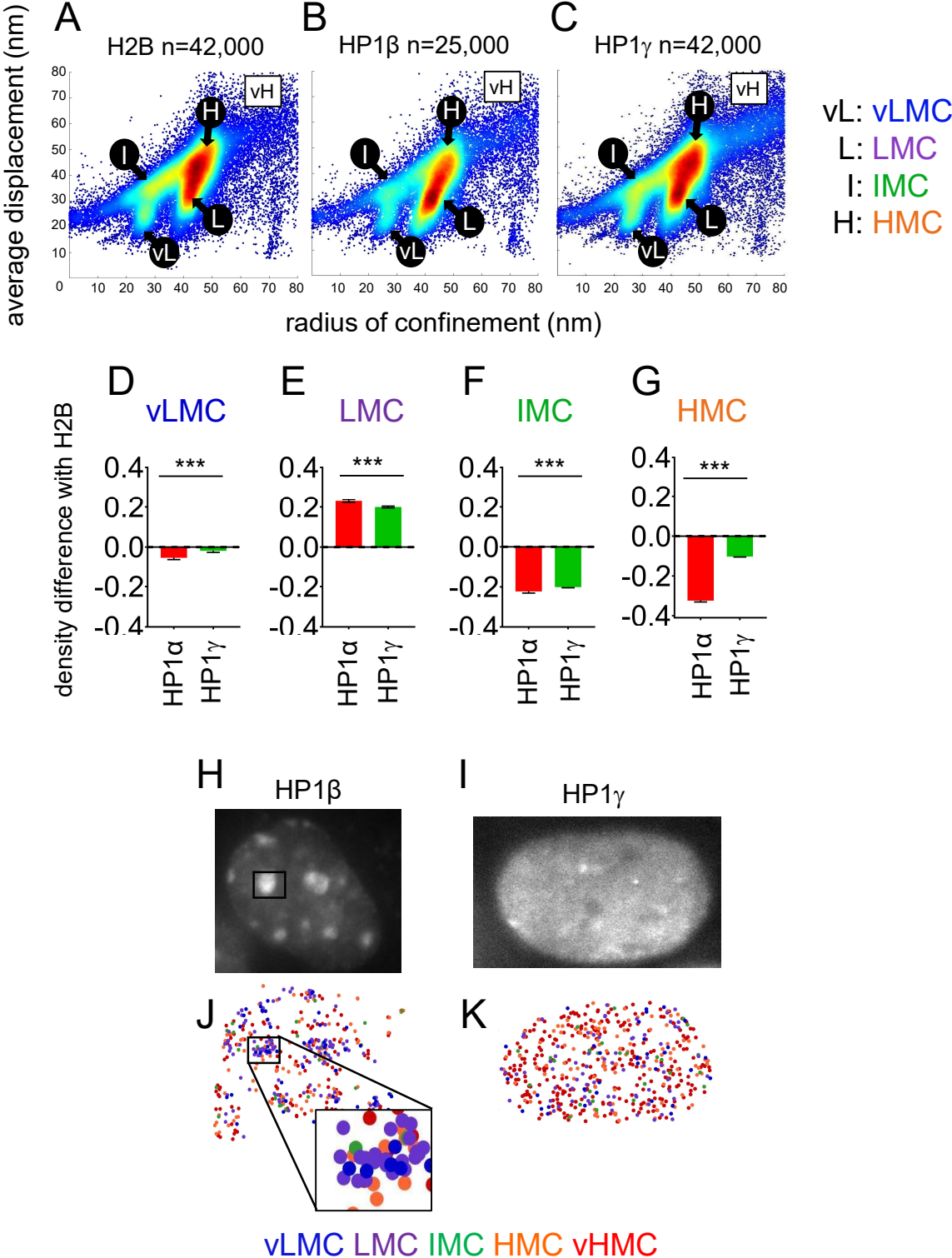
D-G: Relative density levels of HP1 $\beta$  (red) and HP1 $\gamma$  (green) compared to histone H2B in the Low A (D), Intermediate B (E), Intermediate C (F) and High D (G) mobility chromatin.

\*\*\* indicates  $p < 0.0001$ , as determined by one-way ANOVA (see Table S1).

H-I: representative nuclei expressing HP1 $\beta$  (H) and HP1 $\gamma$  (I) showing presence of the heterochromatin regulators at the chromocenters or not.

J-K: Subnuclear localization of HP1 $\beta$  (J) and HP1 $\gamma$  (K) mobility groups

Lerner et al. Supplemental Figure 3



**FIGURE S4 (related to Figure 4): Expression of transcription factors in H2.35 cells**

A: Western blot of FOXA1 in H2.35 cells, showing expression levels near endogenous conditions. FOXA1-HALO : fusion protein; Endog. FOXA1 : Endogenous FOXA1.

Loading control : RNA polymerase II (noted RNA Pol 2).

B: histogram indicating the average fluorescence intensity in arbitrary units of each nuclei imaged by FastSMT, showing overlapping levels. The correspondence of the signals among the different factors with FoxA1 levels, which are within the levels of endogenous FoxA1 (see panel A), indicate that the factors are expressed within a similar range.

C: FRAP recovery curves of the transcription factors of interest, obtained by averaging recovery curves over  $7 < n < 14$  nuclei.

D: Logarithmic frequency distribution of the diffusion coefficients of histone H2B, FOXA1, SOX2, OCT4, KLF4, PU.1, cMYC, GATA4, HNF1A and HNF4A. Red arrow: low-diffusion population, Green arrow: high-diffusion population.

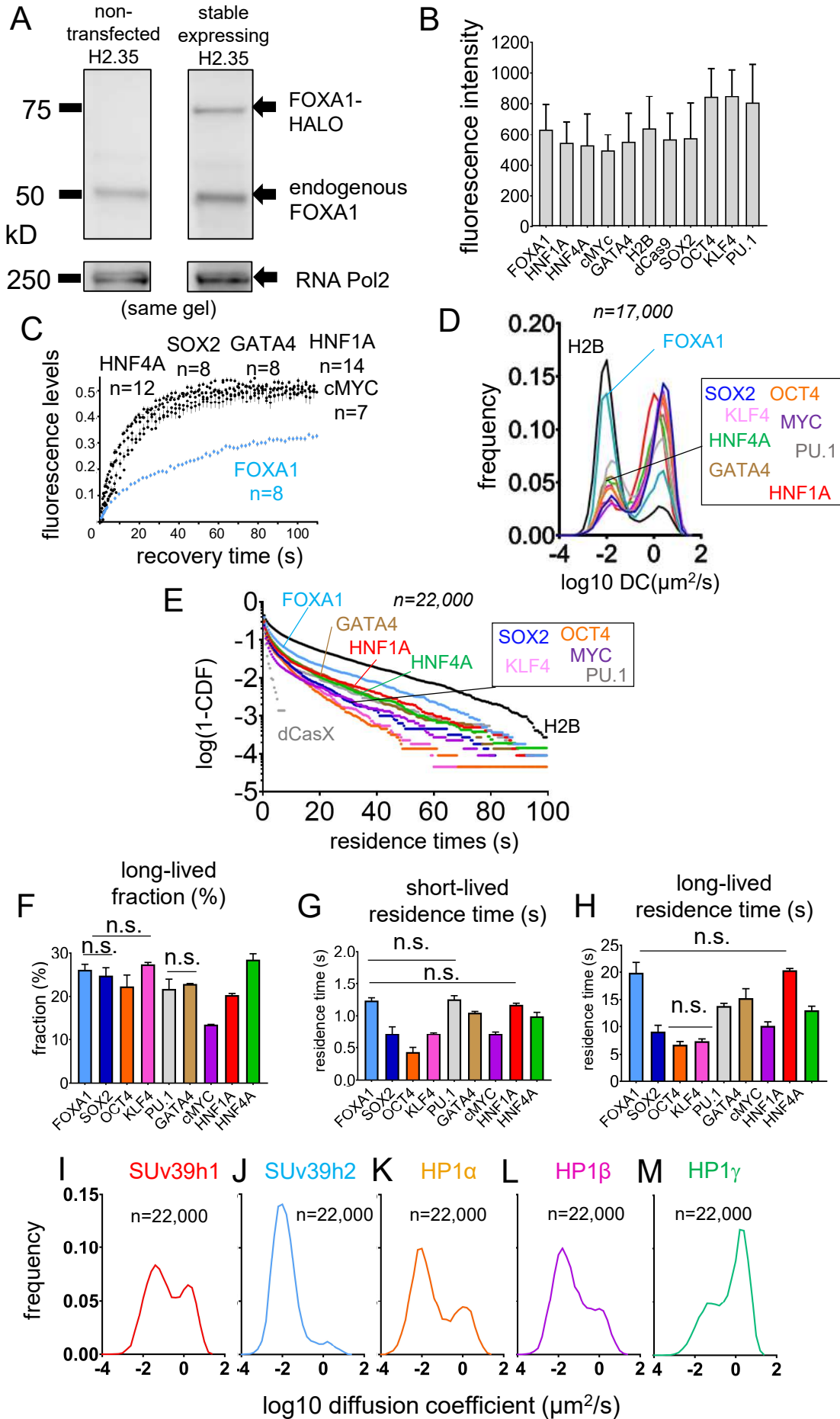
E: Logarithmic frequency distribution of residence times (1-CDF: Cumulative Distribution Function subtracted to 1) for each transcription factor, dCas9X and histone H2B.

F-H: 2-exponential decay fitting of the non-logarithmic residence time frequency distribution provides : Size (in %) of the long-lived fraction (F), and average residence times (in seconds) of the short-lived (G) and long-lived fractions (H) of FOXA1, SOX2, OCT4, KLF4, PU.1, GATA4, cMYC, HNF1A and HNF4A. For graphic simplification, we indicate only non-significant differences (see Table S2).

I-M: Logarithmic frequency distribution of diffusion coefficients for Suv39h1 (I), SUV39h1 (J), HP1 $\alpha$  (K), HP1 $\beta$  (L) and HP1 $\gamma$  (M).



# Lerner et al. Supplemental Figure 4



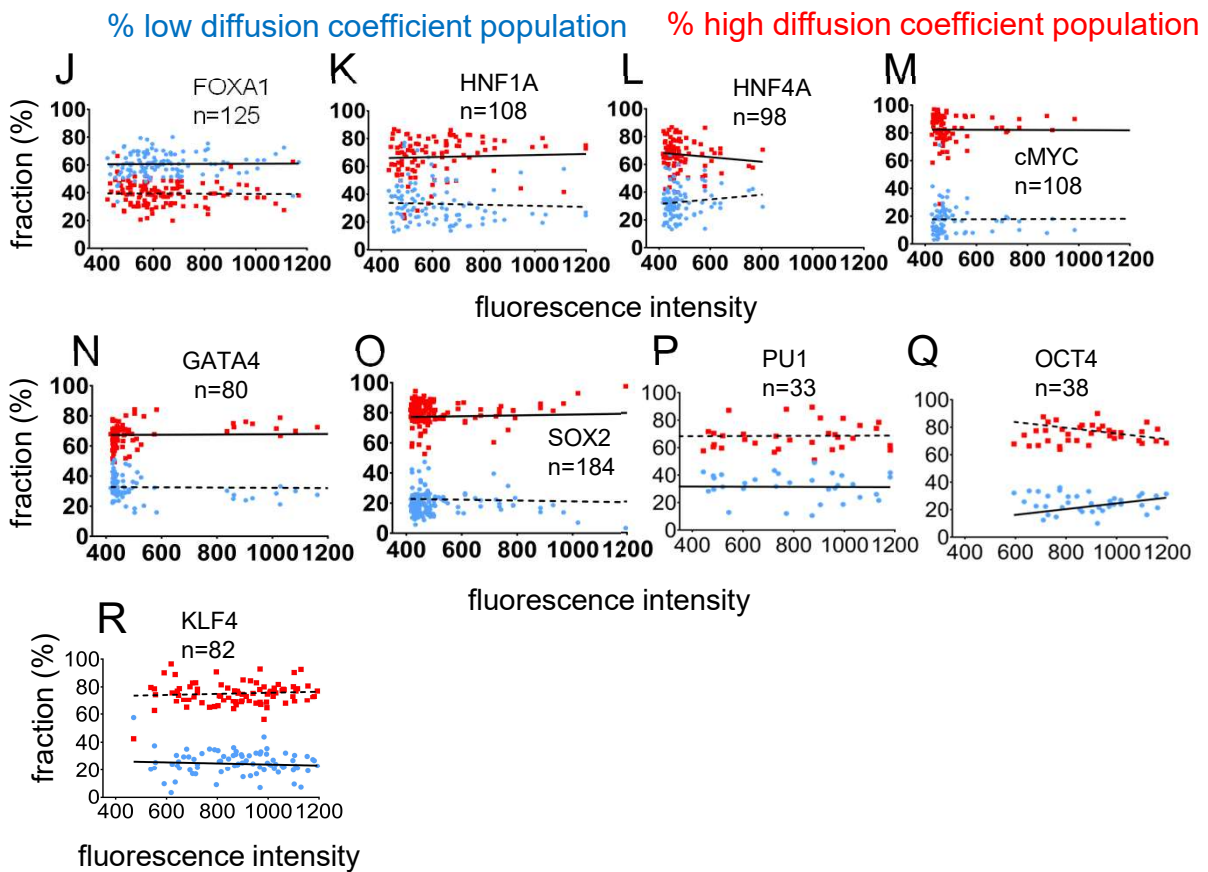
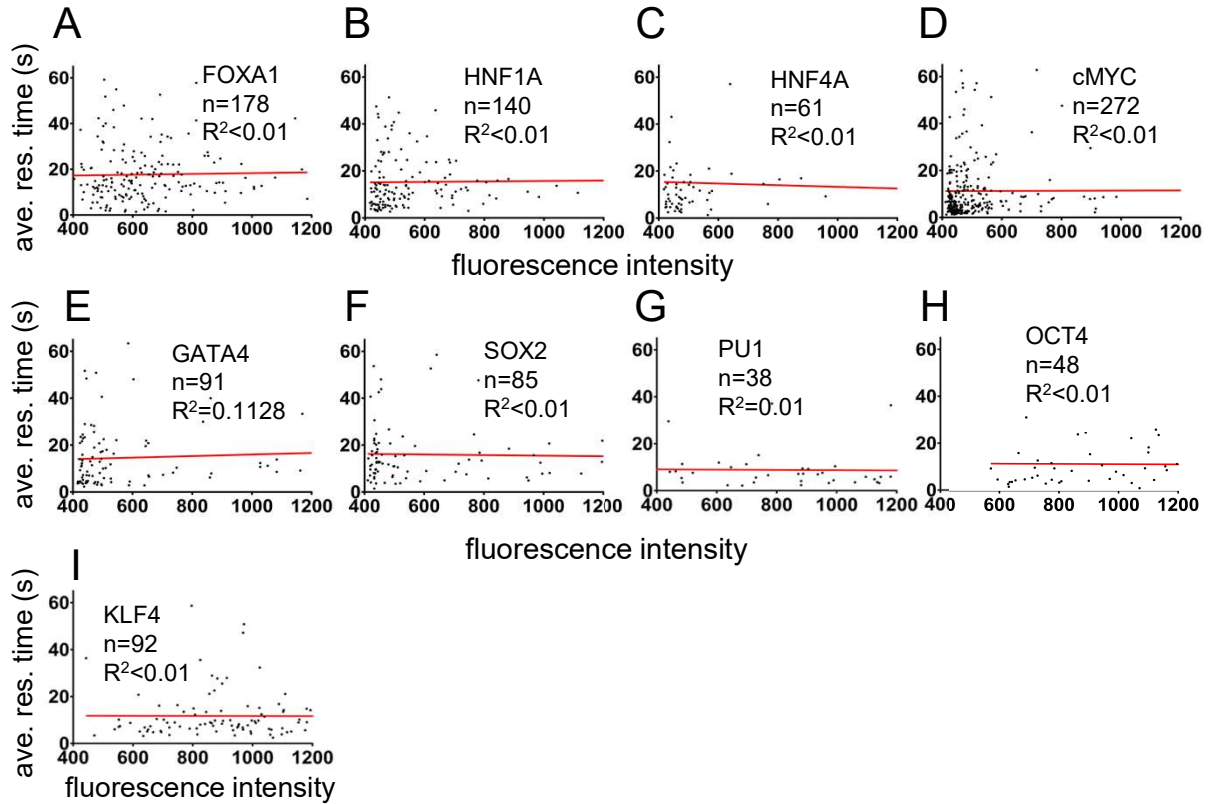
**FIGURE S5 (related to Figure 4): Poor correlation between expression levels, residence time and diffusion of the transcription factors seen in single molecule tracking.**

A-I: Residence time (s) plotted in function of the initial different fluorescence intensity in each imaged individual nucleus.

J-R: size (%) of the low-diffusion (blue) and high diffusion (red) population in function of the initial different fluorescence intensity in each imaged individual nucleus.

S: Distribution of the residence times for each transcription factor, dCas9X and histone H2B. CDF: Cumulative Distribution Function. Fitting was performed by a 2 exponential decay equation.

# Lerner et al. Supplemental Figure 5



**FIGURE S6 (related to Figure 4): Radius of confinement and average displacement discriminate transcription factors with distinct biological roles**

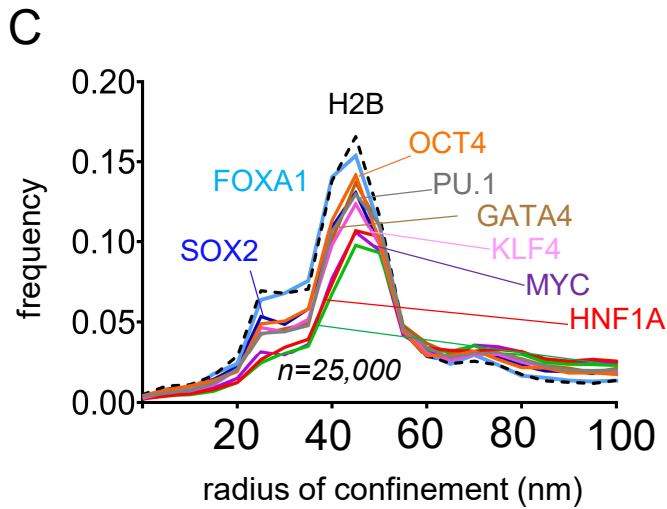
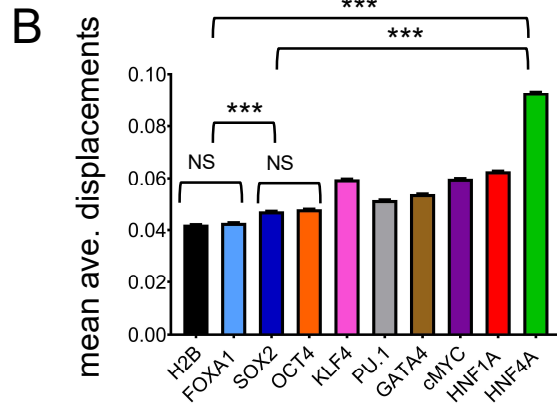
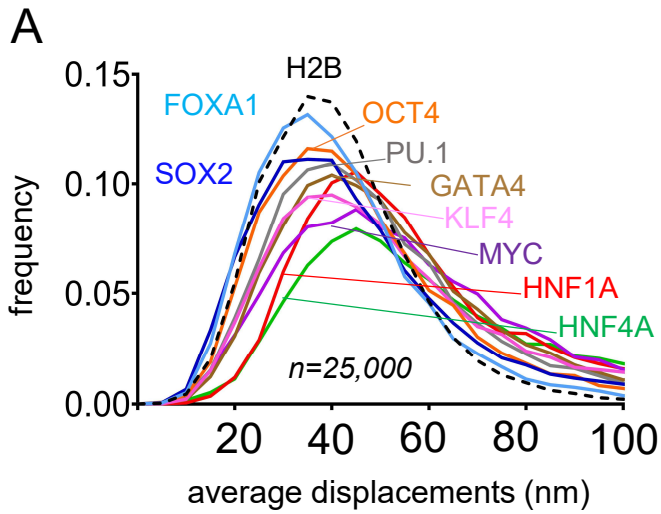
A: frequency distribution of the average displacements for the transcription factors of interest compared to H2B (black dotted).

B: Histogram showing significant mean differences in the average displacement for each factor compared to H2B. p values obtained by one-way ANOVA.

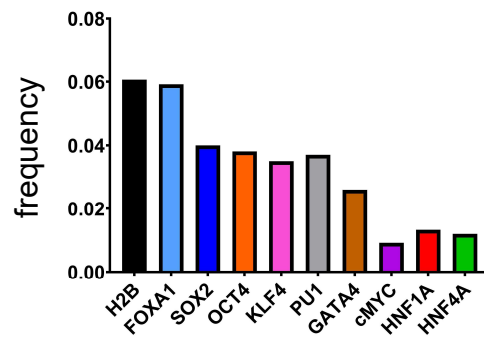
C: distribution of radius of confinement for the transcription factors of interest compared to H2B (black dotted)

D-E: Differences in amplitude of Gaussian 1 (D: 20-40nm) and Gaussian 2 (E: 40-55nm), for each transcription factor and H2B. Differences for Gaussian 3 are not shown, as the population was harder to fit due to high variabilities in the standard deviation of the curve.

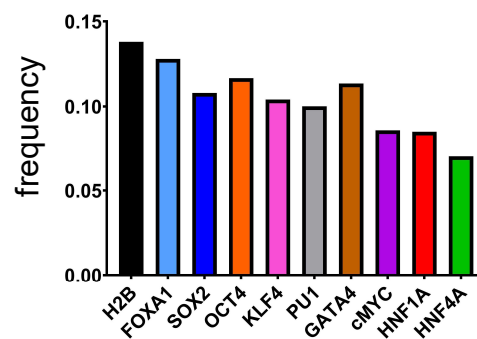
# Lerner et al. Supplemental Figure 6



**D** frequency of RC population 20-40 nm



**E** frequency of RC population 40-55nm



**FIGURE S7 (related to Figure 5): Non-specific DNA binding provides the highest contribution to FOXA1 confinement.**

A: Western Blot of FOXA1 in H2.35 cell stably expressing FOXA1-WT, NHAA and RRAA at similar levels. FOXA1-HALO: fusion protein; Endog. FOXA1: endogenous protein; RNA Pol 2: loading control (RNA Polymerase 2).

B: histogram indicating the intensity of fluorescence of the cells imaged for the Fast and Slow experiments.

C: ChIP of FOXA1-WT, NHAA and RRAA performed with an anti-HALO antibody. Primers amplification at a specific FOXA binding site in the Albumin enhancer and at a FOXA non-bound site the proximity of Nod2 (immune-response gene), as seen in (Iwafuchi-Doi et al., 2016). Averages and standard deviations are obtained over n=3 replicates. For graphical simplification reasons, \*\*\* indicates significant differences between all condition (t-test,  $p < 0.005$ ).

D-F: 2-exponential decay fitting of the non-logarithmic residence time frequency distribution provides : Size (in %) of the long-lived fraction (D), and average residence times (in seconds) of the short-lived (E) and long-lived fractions (F) for FOXA1-WT, FOXA1-NHAA and FOXA1-RRAA. For graphic simplification, we only indicate non-significant differences (see Table S2).

G: Distribution of residence time for H2B showing the 5<sup>th</sup> percentile (above 20s) of the longer residence times, excluding values ranging from 0.5 seconds to 2.5 seconds.

H: Logarithmic frequency distribution of diffusion coefficients for FOXA1 WT (blue), NHAA (red) and RRAA (green). The black and empty arrows indicate low and high diffusing molecules, respectively.

I: Frequency distribution of the radius of confinement for the confined molecules of FOXA1-WT (blue curve), FOXA1-NHAA (red curve) and FOXA1-RRAA (green curve).

J: Frequency distribution of the average displacements for the confined molecules of FOXA1-WT (blue curve), FOXA1-NHAA (red curve) and FOXA1-RRAA (green curve).

K: SDS PAGE gel showing recombinant FOXA1-WT and FOXA1-EKQAAA used for the following EMSA.

L: Electro Mobility Shift Assay with increasing concentration of recombinant FOXA1-WT and FOXA1-EKQAAA, showing the conserved ability of FOXA1-EKQ/AAA to bind DNA in vitro

M: Logarithmic frequency distribution of diffusion coefficients for FOXA1-EKQ/AAA.

# Lerner et al. Supplemental Figure 7

