**A**

HindIII                                       EcoRV

5' GCA|AGCTTCATTCGAGCTCGGTACCCGGGGATCCGAT|ATCGAAGACTCTAAGTACTTTCAATACA***TGCACAGGATGTATATATCTGACACGTGCCTGGAGAC***

***TAGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCCTCGGC***

***ACCGGGATTGTCCAGGGCGGCCGCGTAAGTACTTTCAATACATGCACAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGGCG***

***GTTAAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTGTCCAGGGCGG***

***CCGCGTAAGTACTTTCAATACATGCACAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGGGACAG***

***CGCGTACGTGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTGTCCAGGGCGGCCGCGTAAGTACTTTCAATAC***

***ATGCACAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGCGG***

                                                                        EcoRV

***TGCTAGAGCTGTCTACGACCAATTGAGCGGCCTCGGCACCGGGATTGTCCAGGGCGGCCGCGTAAGACGTCTTCGAT|AT***CCTGCATAATGCATCTGGCA

                                                        SbfI                           HindIII

CGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCA|GGCCTGCAGGCATGCA|AGGGC

ATGCAAGCTTTCATCGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGGAGGGGTCGGCAATTG

                       EFP1                                           EFP2

AACCGGTGCCTAGAGAAGGTGGC<u>GCGGGGTAAACTGGGAAA</u>GTGATGTCGTGT<u>ACTGGCTCCGCCTTTTT</u>CCCGAGGGTGGGGGAGAACCG[TATATAA]

                                   *    Exon 1

TGCAGTAGTCGCCGTGAACGTTCTTTTT[CGCA<u>ACGGGTTT</u>GCCGC<u>CAGA</u>ACAC<u>AGGTA</u>]AGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGG

TTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAG

GCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCT

GTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCT

                                Sp1                                                Sp1

GCACACTGGTATTTCGGTTTTTGGGGCCG<u>CGGGCGGCG</u>ACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGC<u>GAGGCGGGGC</u>CTGCGAGCGCGGCCA

                                                     Sp1           Sp1

CCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTCGGTGCCTGGCCTCGCGCCGCCGTGTATC<u>GCCCCGCCCT</u>GGG<u>CGGCA</u>AGGCTGG

                                                                                  Sp1

CCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGA<u>GCGGGC</u>

<u>GGGTGA</u>GTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGT<u>GACTCC</u>ACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTA

                        Ap1

GTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCA

                                                                           Exon 2

GCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAG[TTTTTTTCTT]CCATT

TCAGGTGTCGTGAGGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGCCACC**ATG 3'**

**B**  5' CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTA 3'

**Figure S1.** The human EF1$\alpha$ promoter driving expression of the eGFP reporter gene. (**a**) The array of four direct repeats of the 177 Widom601 nucleosome positioning sequence [1,2] in pW601-eGFP plasmid is shown in bold italics. The TATA box is in a solid box. The two untranslated exons (Exon 1 and Exon 2) separated by Intron-A of the EF1$\alpha$ promoter are shown in dotted boxes. Binding sites for transcription factors (EFP1, EFP2, Sp1 and Ap1) are noted and underlined [3,4]. The transcription start site is shown by asterisk. The translation start site of eGFP is noted at the 3′ end of the sequence (ATG in bold); (**b**) Sequence of the 100 bp fragment amplified from pUC19 DNA and cloned into the SbfI site between the Widom601 sequence and the EF1$\alpha$ promoter in plasmid pW601-100b-eGFP.
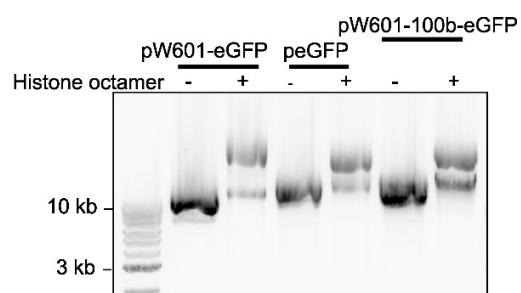
**Figure S2.** Representative analysis of unassembled plasmid DNA or plasmids pre-assembled with unmodified histone octamers. Samples containing 500 ng of plasmid DNA were evaluated on a 0.8% agarose gel in TBE buffer containing 89 mM Tris base, 89 mM Borate and 2 mM Na$_2$EDTA. All detectable plasmid DNA (~98%) had been assembled into chromatin, based on the ~10 ng limit of detection of DNA by ethidium bromide staining. Chromatin assembly was performed as described in our previous studies [5,6].
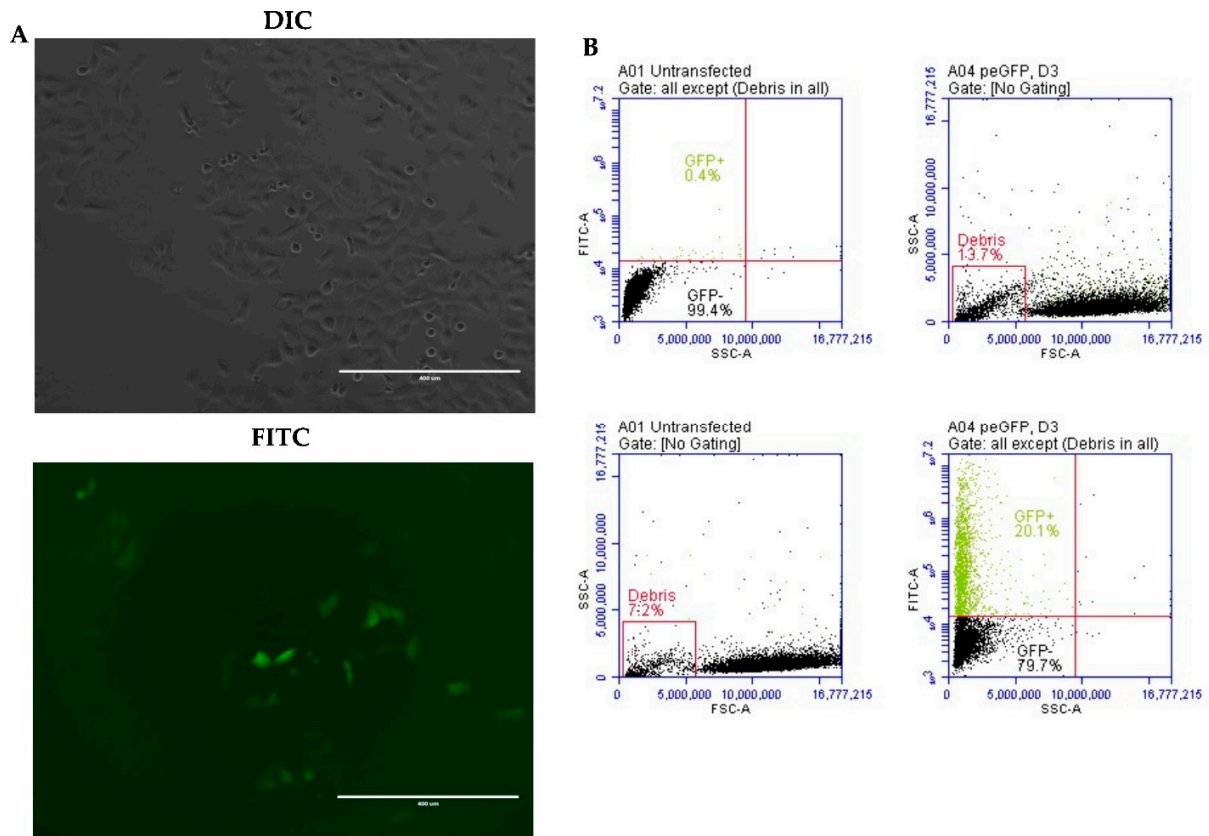
**Figure S3.** Representative fluorescence microscopy images and representative flow cytometry plots. (**a**) 143B cells transfected with unassembled peGFP were imaged at 72 h post-transfection under sterile live cell-imaging conditions using the EVOS FL inverted fluorescence microscope (Life Technologies). Images were taken at 10X magnification. Scale bar equals 400 μm; (**b**) Untransfected 143B cells (left panels) or 143B cells transfected with unassembled peGFP (right panels) were analyzed by flow cytometry at 72 h post-transfection. Cells were trypsinized and $1 \times 10^5$ viable cells were isolated, washed in 1X PBS, and resuspended in 200 μl 1X PBS as described previously. Side scatter (SCC-A) and forward scatter (FSC-A) data were used to assess cell size and complexity. After thresholding to remove "Debris" (top panels) ten thousand events were analyzed, and eGFP+ cells were detected through the FL-1 488 nm channel (FITC-A) against the side scatter (SCC-A). Mean fluorescence intensity of $1 \times 10^4$ was set as the baseline for the FITC-A channel. Untransfected control cells had 0.4% eGFP+ cells above baseline, while those cells transfected with peGFP had a 20.1% eGFP+ cells at three days post-transfection. The fluorescence intensity of eGFP above baseline ranged from $1 \times 10^4$ to $1 \times 10^7$ (arbitrary units)_(Figure S3B, bottom panels), and was used to calculate the mean intensity of fluorescence signal from the eGFP+ cells (see Materials and Methods).
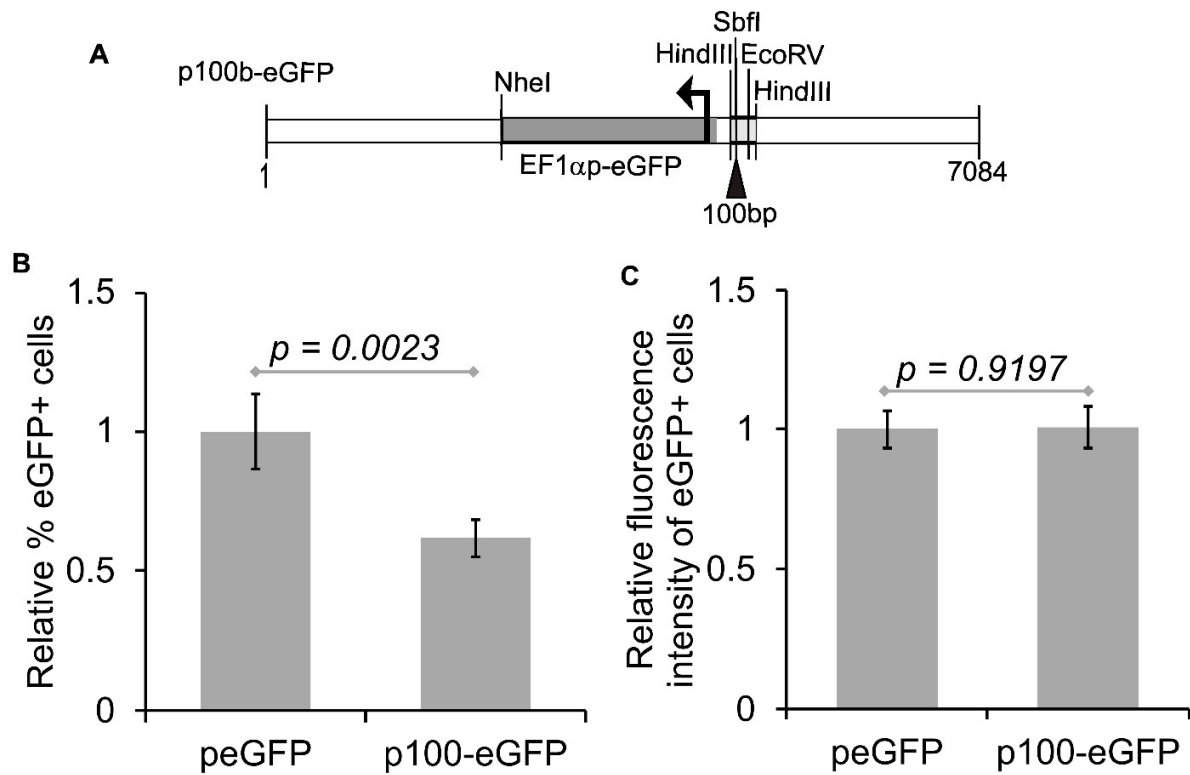
**Figure S4:** Impact of 100 bp spacer on transcription. (**a**) Map of p100-eGFP. (**b**) Percent eGFP+ cells at D3 were calculated for samples transfected with the indicated "naked" plasmids, and then normalized relative to percent eGFP+ cells at D3 from cells transfected with peGFP, which was set to 1 (Mean ± STD, n = 4); (**c**) Mean fluorescence intensity of eGFP+ subpopulations in Figure S2B were calculated and then normalized relative to the mean fluorescence intensity at D3 of cells transfected with peGFP, which was set to 1 (Mean ± STD, n = 4). Statistical significance was determined using One-way Anova [7] and p values are from TukeyHSD post-hoc test [8].
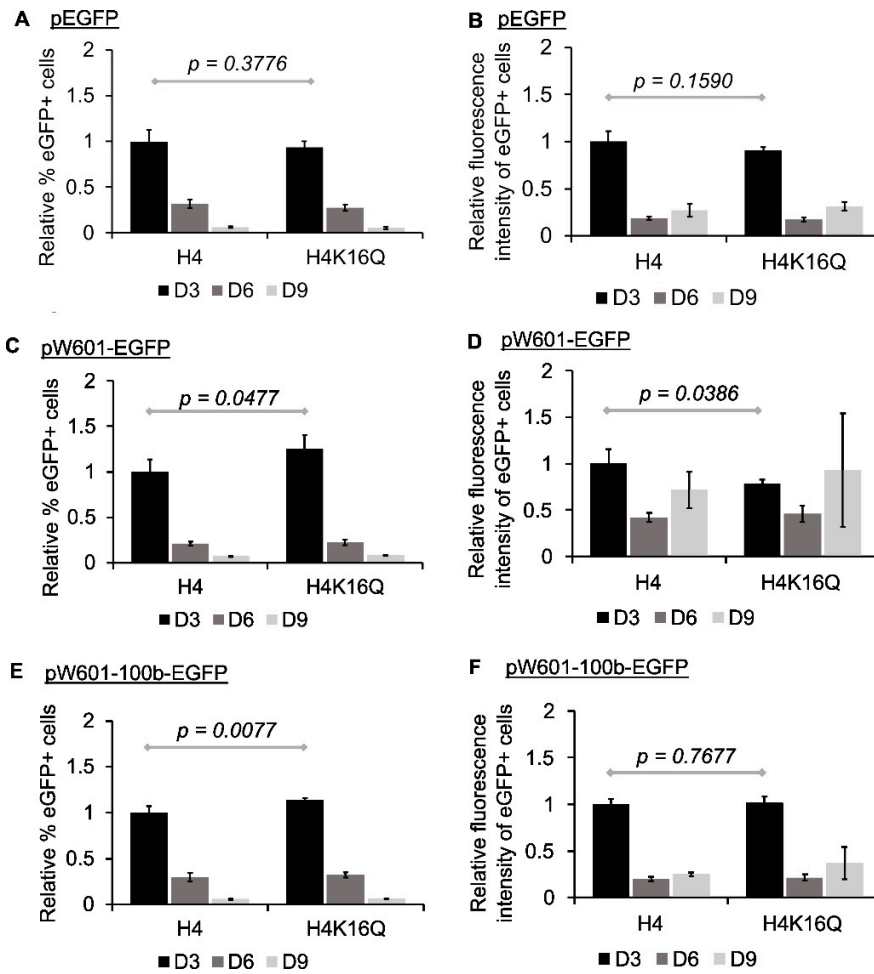
**Figure S5:** The impact of pre-assembled nucleosomes containing H4K16Q relative to unmodified histones (H4) within plasmid constructs. Re-analysis of data from experiment shown in Figure 4. (**a,c&e**) Percent eGFP+ cells from H4K16Q-containing chromatin normalized relative to percent eGFP+ cells from H4-containing chromatin on the indicated plasmids; (**b,d&f**) MFI of the eGFP+ subpopulation from H4K16Q-containing chromatin normalized relative to MFI of the eGFP+ subpopulation from H4-containing chromatin on the indicated plasmids. Statistical significance was determined as in Figure 2.

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