SUPPLEMENTARY METHODS

Expression constructions, cell culture and transfections and immunoprecipitation. The construction of the plasmids encoding C/EBPα was described previously[1]. HP1α was PCR-cloned from a pCMV-SPORT6 vector containing the cDNA for the human HP1α gene (ATCC MGC-4985). The FP fusion proteins were generated using plasmids encoding the monomeric (A206K, ref. 2) forms of ECFP, EYFP (Clontech Takara Bio), and PA-GFP. For cells that expressed PA-GFP-HP1α without a CFP-labeled partner, co-transfection with a plasmid encoding a tandem dimer of monomeric red fluorescent protein (mRFP) was used as a selection marker for the transfected cells[3]. The mouse pituitary GHFT1 cell line[4] was maintained as monolayer cultures in Dulbecco's Modified Eagles Medium containing 10% fetal calf serum. The harvested cells were transfected with the indicated plasmid DNA(s) by electroporation as described previously[5]. For the co-immunoprecipitation assays, whole cell lysates were prepared from GHFT1 cells expressing YFP- HP1α alone or in combination with HA-C/EBPα. The lysates were pre-cleared by incubation with agarose beads, followed by incubation with a HA-specific antibody conjugated to agarose beads (Santa Cruz Biotechnology). The beads were washed several times by centrifugation, and the bound proteins were eluted with denaturing sample buffer (Invitrogen Life Technologies) and analyzed by western blot. The chemiluminescence detection was performed using anti-GFP primary antibody (Molecular Probes, Invitrogen Life Technologies), and a horseradish peroxidase-conjugated anti-rabbit secondary antibody (Pierce Biotechnology).

FRET-FLIM imaging and data processing

The FLIM imaging system consisted of a Nikon TE300 epi-fluorescent microscope equipped with a Plan Fluor 60x NA 1.2 water IR objective lens and a 100W Hg arc lamp. The TE300 was coupled to the Radiance 2100 confocal/multiphoton system and a 10W Verdi pumped, tunable (700 to 1,000 nm) mode-locked ultrafast (78 MHz) pulsed (150 femtosec) laser. The system was equipped with a laser spectrum

analyzer to monitor the excitation wavelength, and a power meter to measure the laser power at the specimen plane. The LaserSharp2000 multiphoton configuration was used to scan the specimen, and the photon-counting module board (SPC-730, Becker & Hickl GmbH, Berlin, Germany) in the Radiance2100 computer matched the X and Y scan synchronizing pulses with the pixel clock signal from control unit, allowing pixel-by-pixel registration of the accumulated photons with the laser scanning. The detector was a fast photomultiplier tube (PMT), with a response time of approximately 150 picoseconds (PMH-100, Becker & Hickl GmbH, Berlin, Germany). The minimum lifetime temporal resolution of the imaging system was approximately 50 picoseconds[6].

The cells expressing the fusion proteins were identified using epi-fluorescence illumination from the arc lamp. The selected cells were then illuminated with the pulsed 840 nm laser line at between 1 and 1.5 mW average power at the specimen plane, and the ECFP-lifetime images were acquired using a 480/40 nm emission filter. A BG-36 glass filter was used to block IR light while transmitting the visible spectrum. The same data acquisition time of 30 sec was used for all FLIM measurements. This resulted in the accumulation of approximately 2000 photon counts on the PMT, which was sufficient to obtain ECFP lifetime measurements. For photoactivation of PA-GFP, the field of view was illuminated for 4s by the arc lamp using a fully refractive mirror and a 405/10 nm barrier filter (Chroma Technology). A second ECFP-lifetime determination was then made under conditions identical to the first measurement. The data analysis software (SPCImage, Becker & Hickl) allowed multi-exponential curve fitting of the acquired data on a pixel-by-pixel basis using a weighted least-squares numerical approach. The lifetime of CFP before and after photoactivation of PA-GFP was analyzed in 10-12 different ROI per cell using a two-component model, and the average mean lifetime (τ_m) for each cell was plotted as a function of the pixel frequency.

SUPPLEMENTARY REFERENCES

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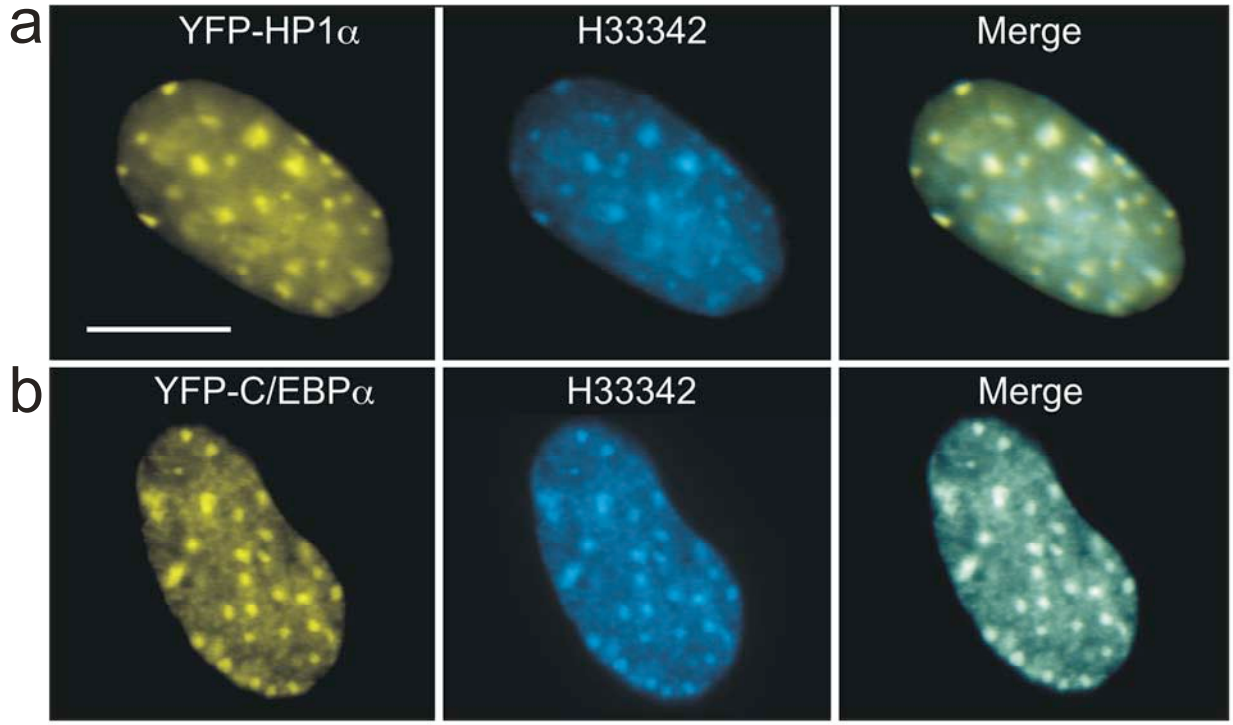
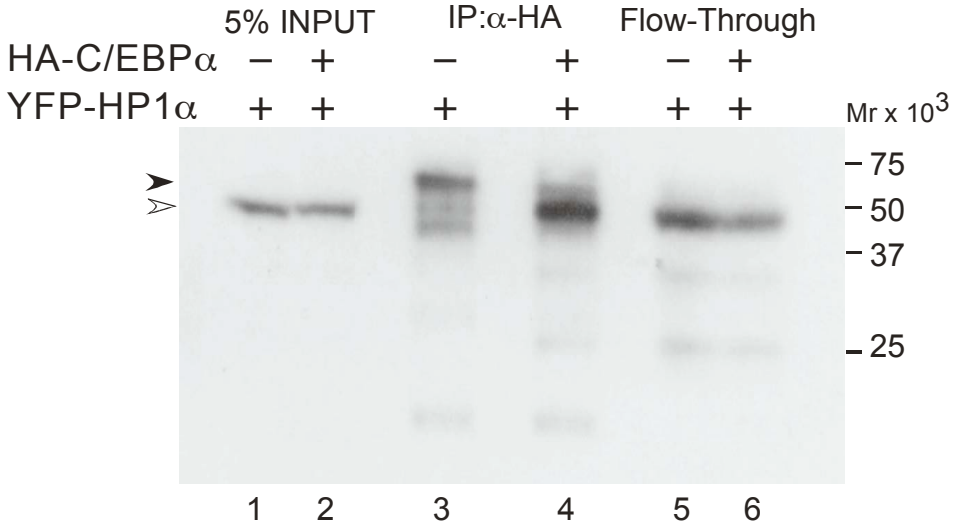
Supplementary Figure 1



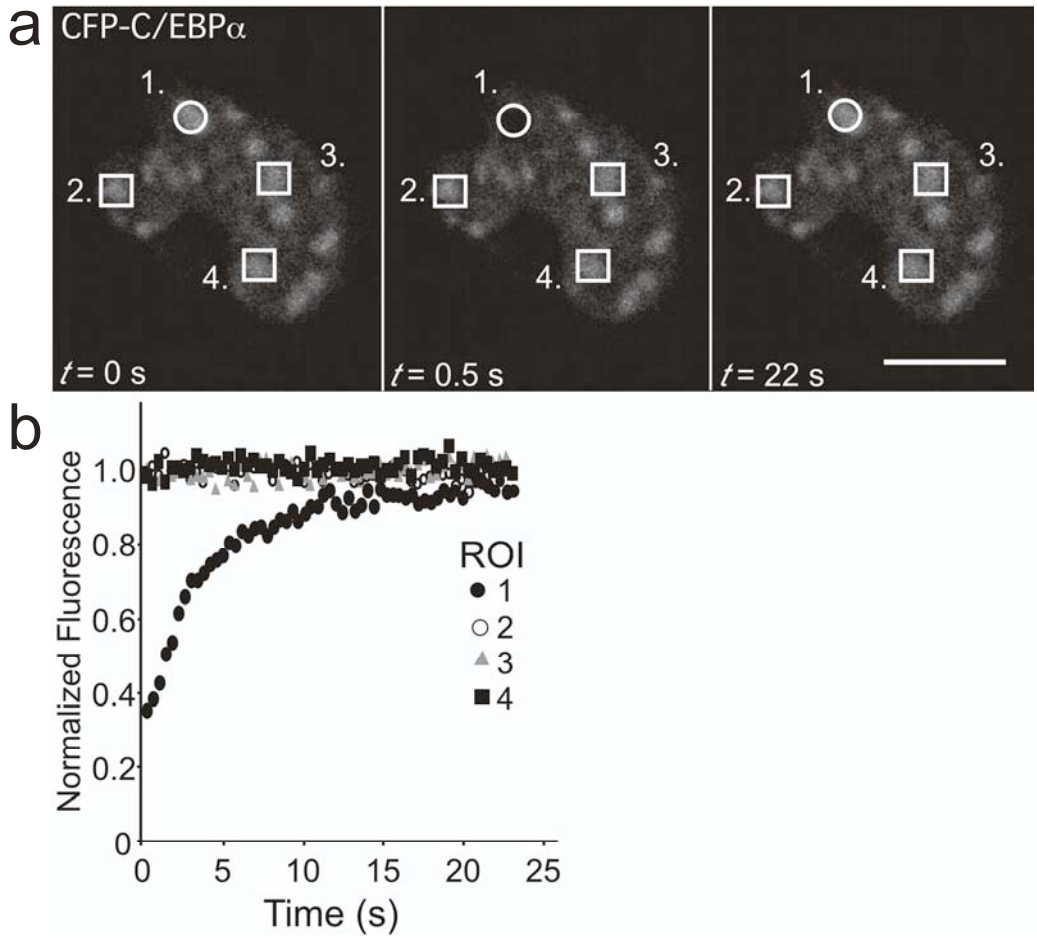
Supplementary Figure 1. Wide-field microscope images showing the nuclei of mouse GHFT1 cells that expressed either **(a)** YFP-HP1α or **(b)** YFP-C/EBPα, the calibration bar is 10 μm. In each case, the living cells were stained briefly with the cell permeant DNA-dye, H33342, before imaging. The merged images show that both YFP-HP1α and YFP-C/EBPα localized to the centromeric heterochromatin preferentially stained by H33342.

Supplementary Figure 2



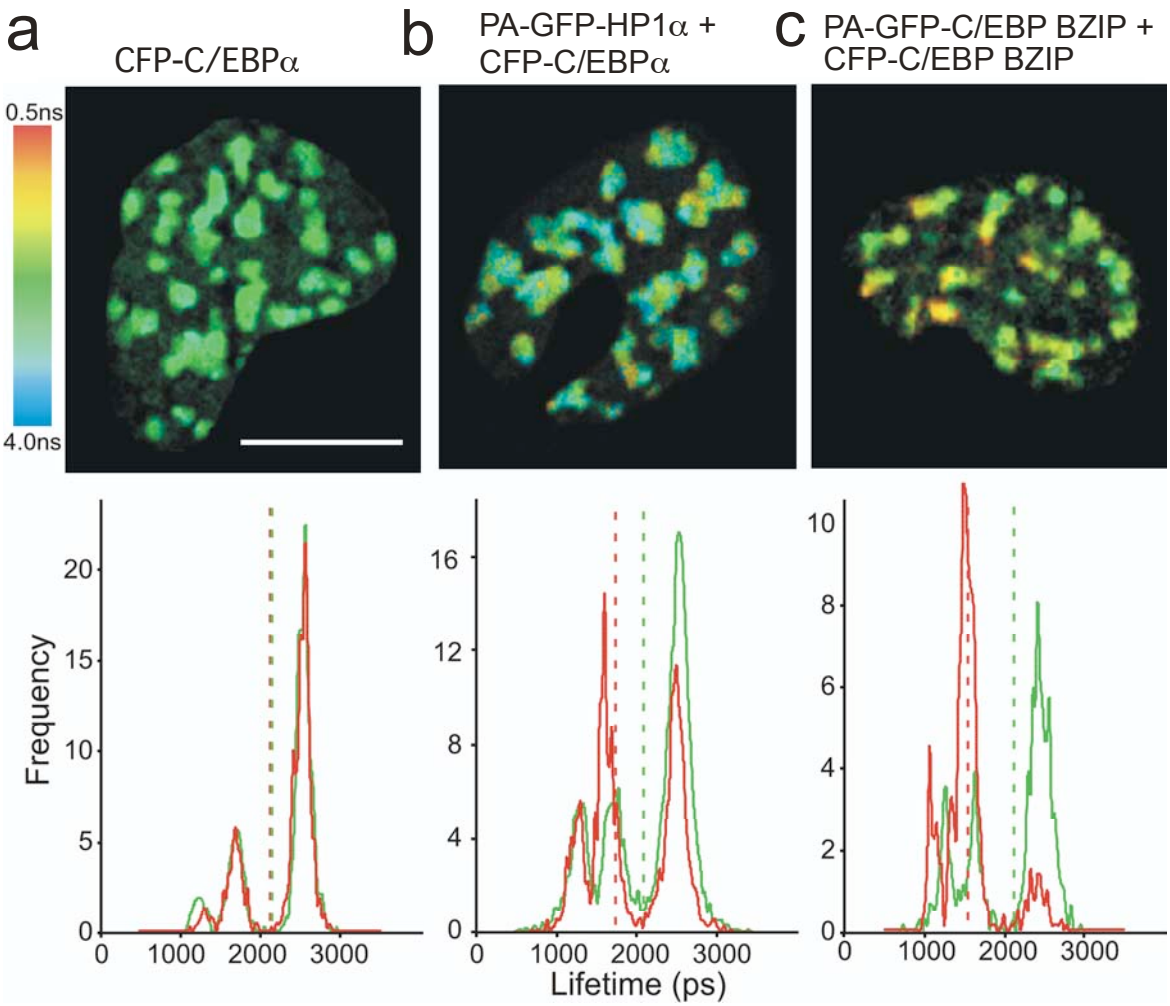
Supplementary Figure 2. The full blot from the co-immunoprecipitation analysis of the association of HP1α and C/EBPα. The lysates from cells expressing YFP-HP1α alone or in combination with HA-tagged C/EBPα were incubated with a HA-specific antibody conjugated to agarose beads, which were then washed and the bound proteins were eluted with denaturing sample buffer. The input lanes (1,2) are 5% of the total protein loaded for IP, and the YFP-labeled HP1α (~ 48 KD) was detected by western blot (WB) using an anti-GFP antibody (open arrowhead, lane 4). The flow-through lanes show the unbound fraction of YFP-HP1α (lanes 5,6). The filled arrowhead indicates a higher molecular weight (~ 60 KD), non-specific band detected in the immunoprecipitates.

Supplementary Figure 3



Supplementary Figure 3. Control experiments monitoring the photobleaching of CFP under conditions used for the photoactivation of PA-GFP. **(a)** A 500-ms pulse of 405-nm light at 135 μW laser power was delivered to the nucleus of a cell expressing CFP-C/EBPα (circle), and the CFP signal was monitored at that site, as well as three other ROI (squares) over a 22-s time frame, the calibration bar is 10 μm. (**b**) Measurement of the changes in CFP fluorescence at the site of photoactivation (ROI 1) and for the other three ROI shown in **(a)**.

Supplementary Figure 4



Supplementary Figure 4. The mean donor lifetime distributions obtained by two-component analysis of CFP fluorescence lifetime from cells expressing: **(a)** CFP-C/EBPα; **(b)** CFP-C/EBPα and PA-GFP-HP1α; **(c)** CFP-C/EBP BZIP and PA-GFP-CEBP BZIP. The top panels show the two-photon excited fluorescence lifetime images of the nuclei from cells expressing the indicated proteins following the photoactivation of PA-GFP by a 405-nm light pulse. The lookup table shows the range of mean lifetime distributions, with orange indicating 0.5 ns and cyan indicating 4.0 ns, the calibration bar is 10 μm. The bottom panels show the corresponding mean lifetime distributions for the donor, determined from 10 different ROI in each cell nucleus and plotted as a function of pixel frequency. The 2.5-ns peak corresponds to the unquenched natural lifetime of CFP measured before (green curves) the photoactivation pulse. The lifetime for CFP-C/EBPα expressed alone (**a** and corresponding plot) was unchanged by the photoactivation pulse (red curves). In contrast, CFP-C/EBPα **(b)** and CFP-C/EBP BZIP **(c)** showed shifts to shorter lifetimes (1.2-1.6 ns) upon photoactivation of the PA-GFP-labeled protein partner, denoting quenched donor lifetime components. The dotted lines indicate the mean lifetime values before (green) or after (red) the photoactivation pulse.