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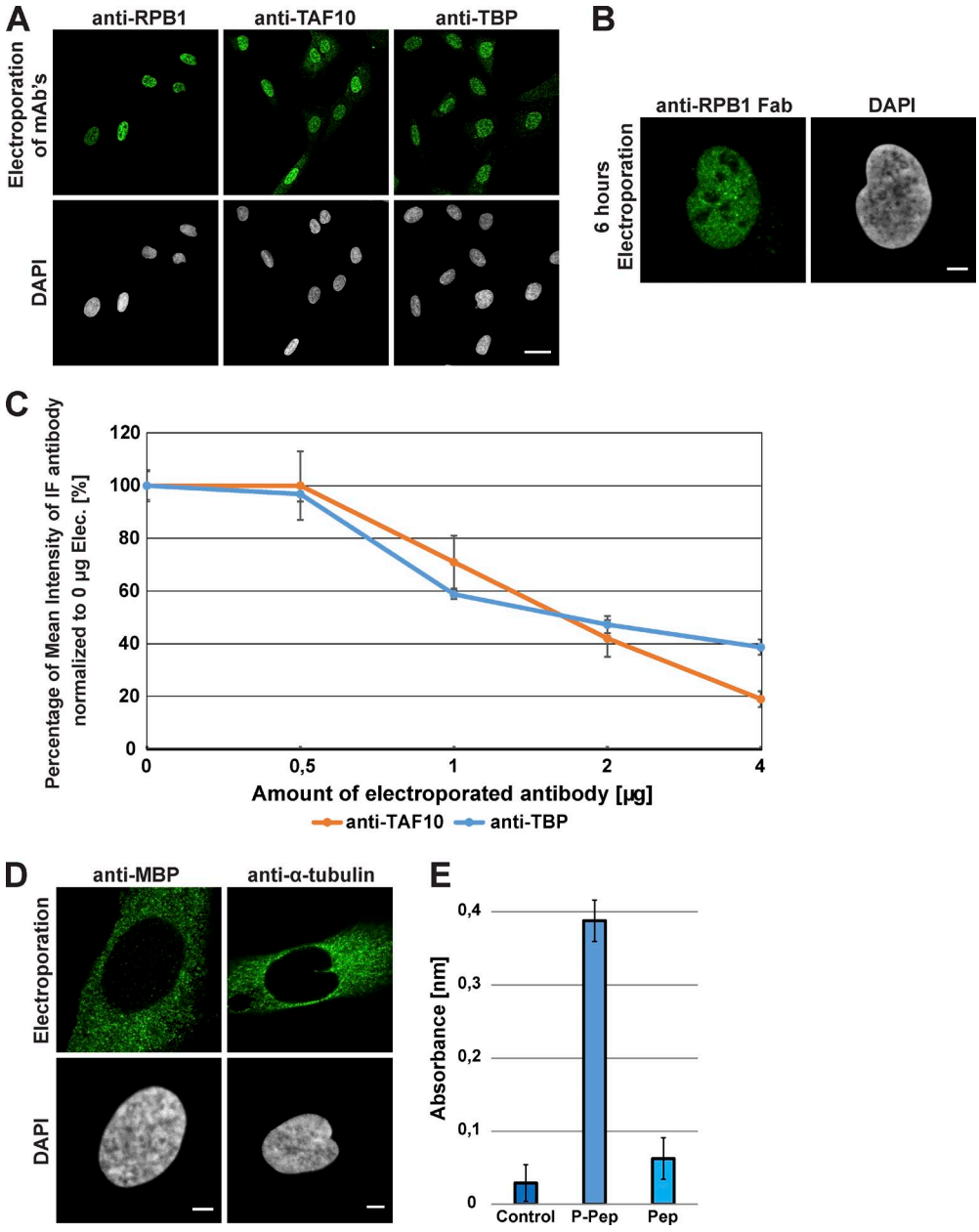


Figure S1. **Efficiency, localization, target binding, and affinity of different antibodies by using VANIMA. (A)** The Alexa Fluor 488–labeled mAbs binding specifically to the transcription factors RPB1, TAF10, and TBP were transduced in U2OS cells, and their localization in the cells was monitored 24 h after treatment (see also Fig. 2 A). Bar, 30 µm. **(B)** The Alexa Fluor 488–labeled Fab fragment against RPB1 was transduced in U2OS cells and monitored by confocal microscopy 6 h after electroporation. Bar, 5 µm. **(C)** Quantification of the competition assay shown in Fig. 2 C. Alexa Fluor 488–labeled antibodies against TAF10 (anti-TAF10) or TBP (anti-TBP) were transduced into U2OS cells in increasing amounts. To verify binding of the antibodies to their target, a competition assay was performed afterward by adding a constant amount (2 µg) of the same antibody, but Alexa Fluor 568–labeled as IF antibody, after fixation. The graph shows the mean fluorescence intensity of the nuclei labeled with the IF antibody for each condition. The anti-TAF10 and anti-TBP measurements were done 24 or 48 h after transduction, respectively. The percentage of mean intensity was normalized to the 0-µg transduction. Error bars represent the SD obtained with 10 recorded cells for each condition. **(D)** Antibodies against MBP or α-tubulin were transduced into U2OS cells, and their localization in the cells was monitored 24 h after electroporation. Bar, 5 µm. **(E)** Analysis of the binding specificity of the anti-γH2AX Fab by ELISA. The ELISA plate was coated with either the nonphosphorylated (Pep) or the phosphorylated peptide (P-Pep) corresponding to the C-terminal end of H2AX (see Materials and methods). After addition of 1 µg/ml anti-γH2AX Fab and subsequent washing, bound Fab was revealed with HRP-labeled anti-mouse immunoglobulins. The histogram shows the mean value of the absorbance measured in several wells after hydrolysis of the HRP substrate and error bars indicate the SD of the measurements. Control, no coating. All images in this figure are showing typical nuclei after fixation and counterstaining with DAPI as well as single z sections.

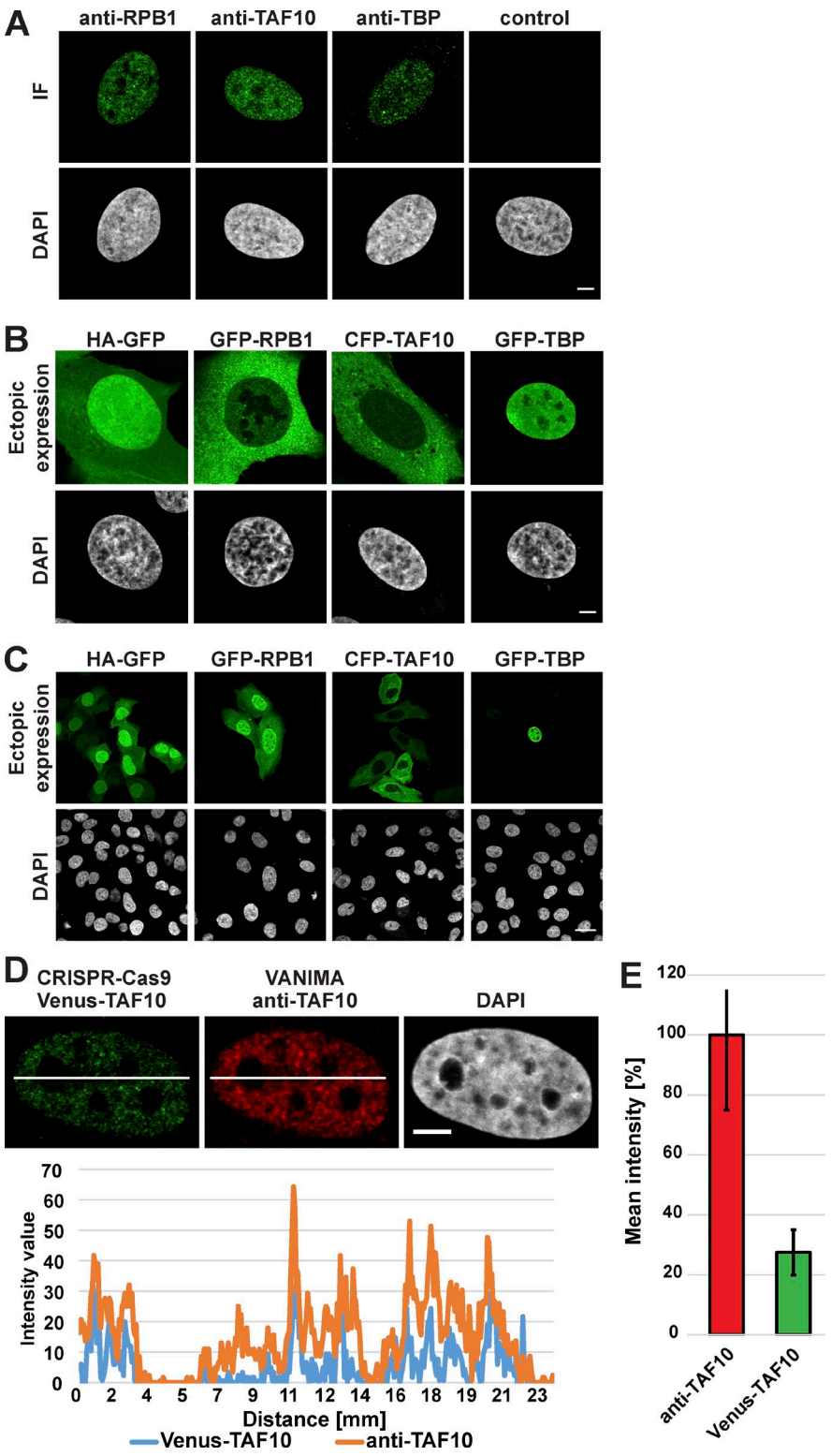
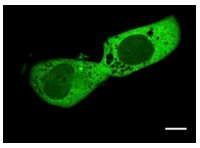
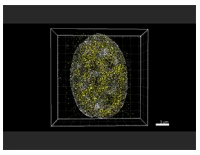


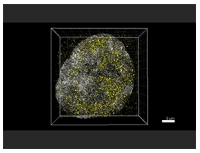
Figure S2. **Imaging transcription factors with classical IF or after genetic tagging with fluorescent tags by using confocal microscopy.** All images show single z sections of the nuclei. **(A)** The endogenous transcription factors were detected in fixed U2OS cells with the indicated antibodies by classical IF. Control: Cells treated only with Alexa Fluor 488–labeled secondary antibodies. Bar, 5 µm. **(B)** The indicated fluorescently tagged transcription factors were visualized 48 h after transfection with the plasmids expressing the indicated fusion proteins. Bar, 5 µm. **(C)** Same as in B, but cells were observed with lower magnification. Bar, 30 µm. **(D)** Detection of electroporated Alexa Fluor 568–labeled anti-TAF10 mAb (red) in CRISPR/Cas9-modified U2OS cells stably expressing Venus-TAF10 (green). The area taken for the intensity profile measurements is indicated by white lines in the nuclei corresponding to the Venus-TAF10 and anti-TAF10 images. An intensity profile of the Venus-TAF10 (blue) and anti-TAF10 Alexa Fluor 568 (orange) signal is shown under the confocal images. Bar, 5 µm. **(E)** Quantification of the mean intensity of nuclei either transduced with Alexa Fluor 488–labeled anti-TAF10 or expressing Venus-TAF10. The percentage of mean intensity was normalized to the anti-TAF10 transduced sample. Error bars represent the SD obtained with 10 recorded cells for each condition. All micrographs correspond to typical nuclei observed in each case after counterstaining with DAPI.



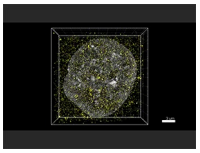
Video 1. **Transport of the labeled anti-RPB1 mAb from the cytoplasm into the nucleus of living cells (see Fig. 1 A).** U2OS cells were transduced with Alexa Fluor 488–labeled anti-RPB1 antibody and incubated for 6 h before starting image acquisition. Imaging was performed on a confocal microscope focusing on one single z plane and by taking one image every hour. Total time of analysis: 20 h. Bar, 15 µm.



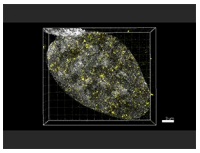
Video 2. **Analysis of a nucleus of U2OS cells transduced with the labeled anti-RPB1 mAb (yellow) by 3D-SIM super-resolution microscopy (see Fig. 4 A).** Images were taken 24 h after transduction and correspond to a full z stack of the whole nucleus. The video represents a typical nucleus recorded after fixation of the cells and subsequent counterstaining with DAPI (gray). Bar, 3 µm.



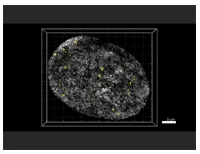
Video 3. **Analysis of a nucleus of U2OS cells transduced with the labeled anti-TAF10 mAb (yellow) and counterstaining with DAPI (gray) by 3D-SIM microscopy (see Fig. 4 A).** Images were taken as indicated in the legend of Video 2. Bar, 3 µm.



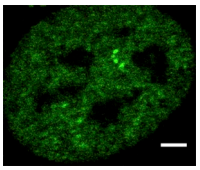
Video 4. **Analysis of a nucleus of U2OS cells transduced with the labeled anti-TBP mAb (yellow) and counterstaining with DAPI (gray) by 3D-SIM microscopy (see Fig. 4 A).** Images were taken as indicated in the legend of Video 2. Bar, 2 µm.



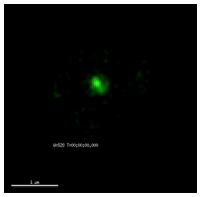
Video 5. **Analysis of U2OS nuclei after transduction with labeled anti-γH2AX Fab (yellow) in the presence of HU treatment by 3D-SIM microscopy (see Fig. 6 A).** The images correspond to a full z stack of a typical nucleus recorded in each case after cell fixation and counterstaining with DAPI (gray). Bar, 3 µm.



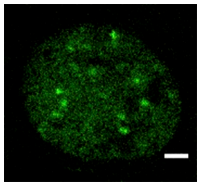
Video 6. **Analysis of U2OS nuclei after transduction with labeled anti-γH2AX Fab (yellow) in the absence of HU treatment by 3D-SIM microscopy (see Fig. 6 A).** The images correspond to a full z stack of a typical nucleus recorded in each case after cell fixation and counterstaining with DAPI (gray). Bar, 3 µm.



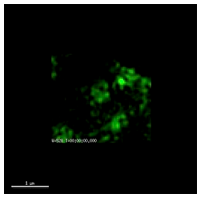
Video 7. **Live-cell imaging of RNA Pol II after transduction of labeled anti-RPB1 mAb (see Fig. 7 A).** 24 h after electroporation in the presence of Alexa Fluor 488–labeled anti-RPB1 mAb, the U2OS cells were analyzed by confocal microscopy. The nuclei were imaged by focusing on one z section and over a period of 2.5 h. The pictures were taken every 10 min. Bar, 3 µm.



Video 8. **3D-SIM live-cell imaging of one distinct RNA Pol II cluster after transduction of labeled anti-RPB1 mAb into U2OS cells (see Fig. 7 B).** The dynamics of a RNA Pol II cluster were analyzed 24 h after electroporation with Alexa Fluor 488–labeled anti-RPB1 mAb by using 3D-SIM microscopy. The video shown is a maximum-intensity projection of a 1-µm z stack. The nuclei were imaged over a period of 45 s, and pictures were taken every 4.1 s. Bar, 1 µm.



Video 9. **Confocal live-cell imaging of γH2AX foci (see Fig. 7 C).** 24 h after electroporation, U2OS cells transduced with Alexa Fluor 488–labeled anti-γH2AX Fab were analyzed by confocal microscopy after treatment with NCS. The nuclei were imaged by focusing on one z section over a period of 4 h. Pictures were taken every 10 min. Bar, 5 µm.



Video 10. **3D-SIM live-cell imaging of a γH2AX foci by using VANIMA (see Fig. 7 D).** U2OS cells transduced with Alexa Fluor 488–labeled anti-γH2AX Fab were treated with NCS and analyzed by live-cell imaging by using 3D-SIM microscopy. The first time point was acquired 10 min after drug treatment. The video shown corresponds to a maximum-intensity projection of a 1-µm z stack. The nuclei were imaged over a period of 45 s, and pictures were taken every 15 s. Bar, 1 µm.

Table S1. **Efficiency of anti-RPB1 mAb delivery**

Cell line	Efficiency	Viability
	%	%
U2OS	99	92
HFF-1	99	99
mES cells	97	56
S2 cells	94	71

The indicated cell lines were electroporated in the presence of the Alexa Fluor 488–labelled anti-RPB1 mAb (2 µg). The efficiency (%) was calculated by counting 100 cells and determined the percentage of cells showing a positive nuclear staining 24 h after transduction. The percentage of viability corresponds to the number of live cells after the electric treatment normalized to the number of living cells in the electroporation mixture. All counts were performed in the presence of Trypan blue. HFF-1, human foreskin fibroblast cells; mES, mouse embryonic stem.

Table S2. **Validated primer pairs used for the quantification of Pol II pre-mRNA as well as Pol I (*RN18S*) and Pol III (*RN7SK, RPPH1*)**

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
CCT4	AGAGCACTGACTGATACCAACAGA	AGACACTAAAAGCAACTTGTGCTG
EEF2	CGACTCTTCACTGACCGTCTC	TGTGTGTAAGGTCACCTCTTTCTC
EIF3L	CTGGATGGTGAATTTCAGTCAGC	AACACTTAATACAAGACCCCAAGC
GAPDH	CTCACATATTCTGGAGGAGCCTC	TTACCAGAGTTAAAAGCAGCCCT
GMPS	GGAGAGAGGGCATAGACCTTGT	AGCATACACAGAATTAGGTCCTCC
GNB1	ATCTCCAGTGTGTCCGGTAAAC	ACCCAAGAAGTTAAGGCTGATGTC
MYBL2	CAGGTGGATGTGAAGGGCTATG	TGTGCCATACTTCTTAACCAGCT
PKM	CAAAGCTTTCCGTGGCTGTG	GAGCTGGATTCTAGTGTGGGAG
RBBP5	AGACAATGCTCCCAATGTGTC	AGGTTTACCTCTGGAAGGATCAG
RN18S	AAACGGCTACCACATCCAAG	GGCCTCGAAAGAGTCCTGTA
RN7SK	CGGTCAAGGGTATACGAGTAGC	TTGGAAGCTTGACTACCCTACG
RPL8	ACGATTGTACCCTCAGGCATG	CGCATTGTTTCTTACTGTGCTGA
RPPH1	GGCGGAGGAGAGTAGTCTGAAT	CGGAGCTTGGAACAGACTCA
RPS18	CCTTATCGGCCTTACTGTTTGAT	AAATATGCTGGAACTTTTCAGGG
SF3B2	CACCTGTATCTTTTGTTTCCGCTT	CAGTGAAGAGCTGAGGTGTCTC
TPM2	AAATGGGATGAGAAGGTACAGGAC	GGAGAAAACCATCGATGACCTAGA

Transcripts shown in Fig. 3 A.