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Supplemental information

ATAC and SAGA co-activator complexes utilize co-translational assembly, but their cellular localization properties and functions are distinct

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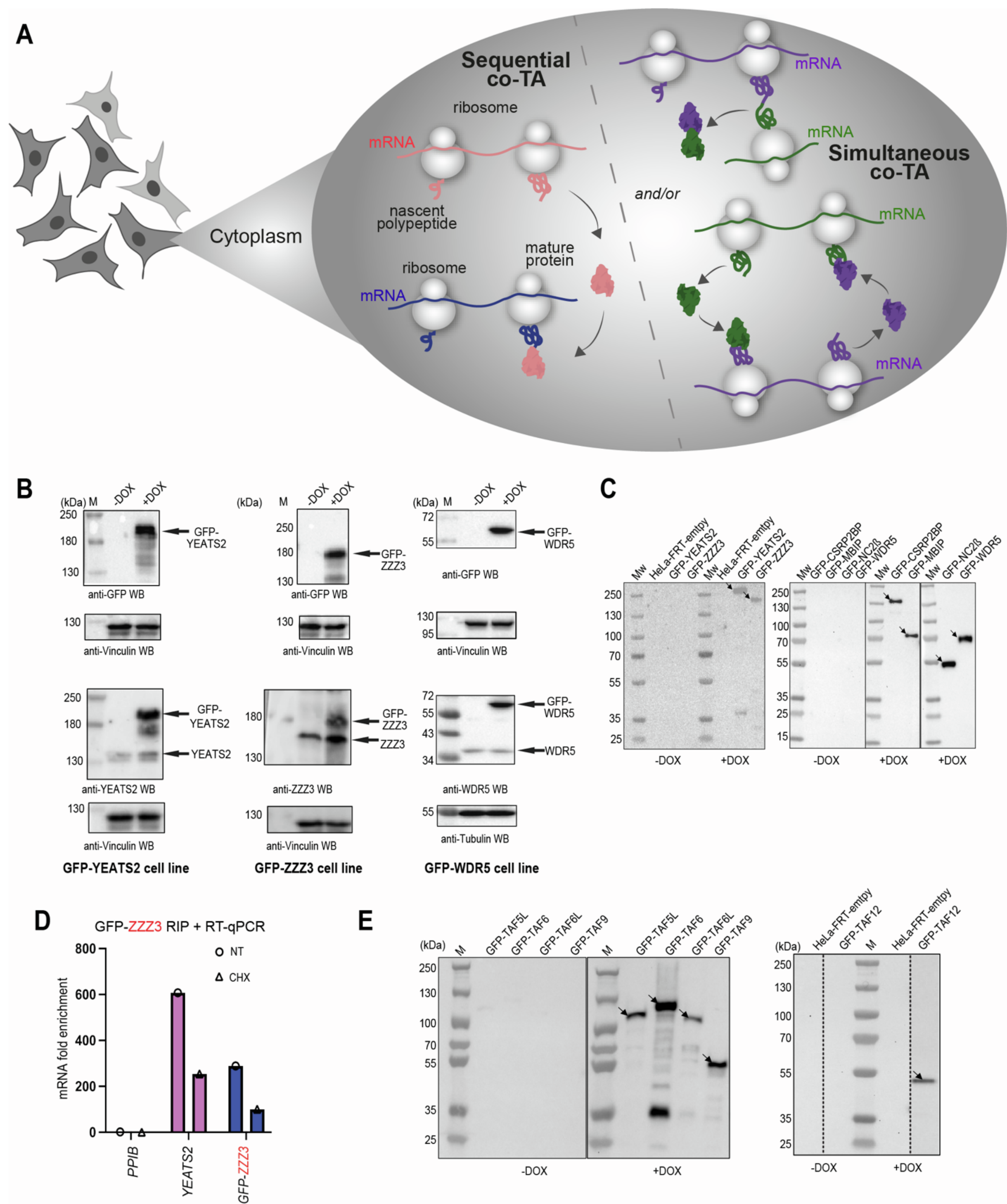
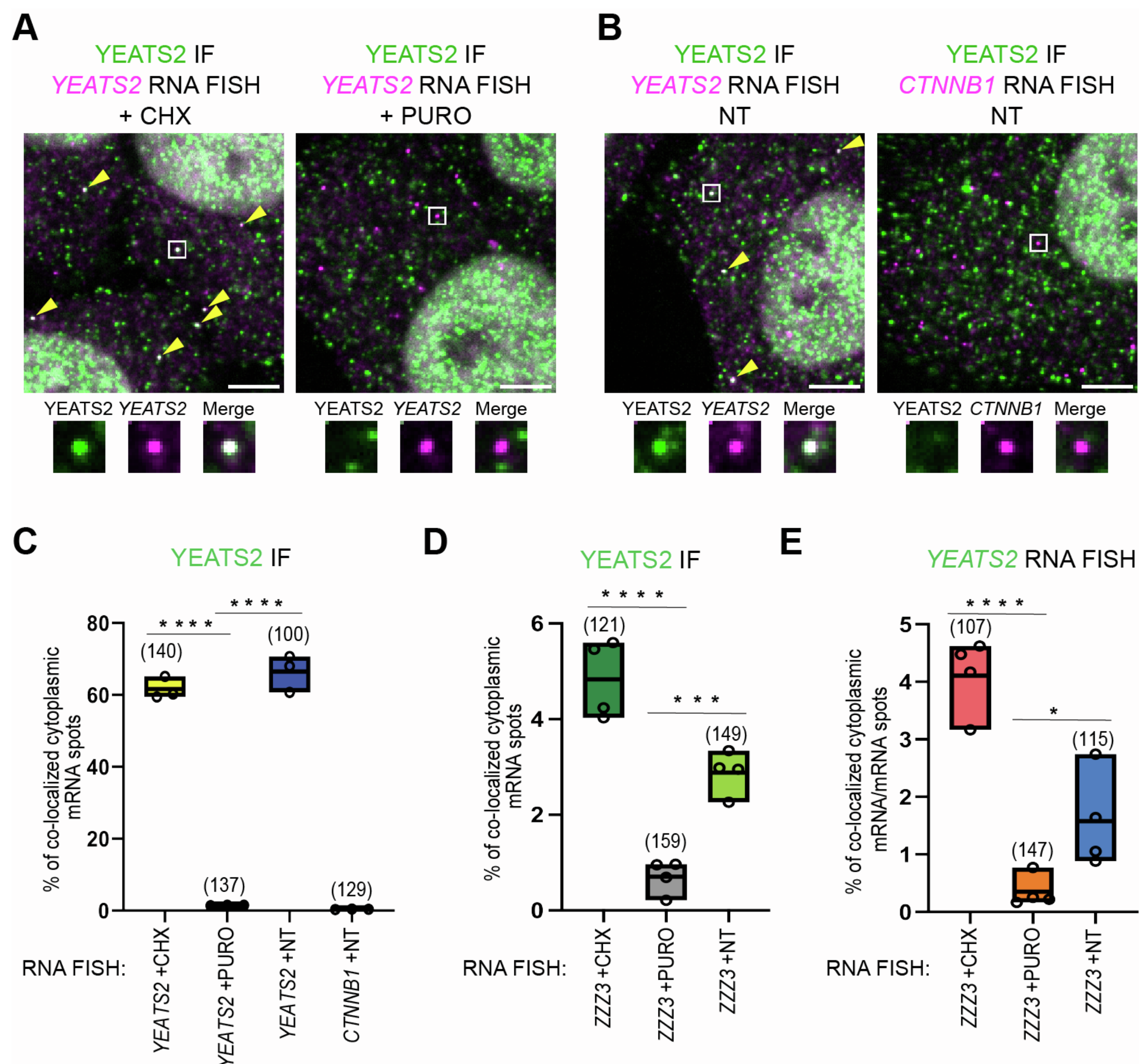
Supplemental Figures, Tables and their legends



Supplemental Figure 1. Co-translational assembly of the ATAC core module.

(**A**) Illustration of simultaneous and sequential co-translational assembly (co-TA) pathways in the cytoplasm of mammalian cells. In the case of sequential co-TA (left side) a fully synthetized protein binds to the nascent protein partner during translation. In the simultaneous co-TA model

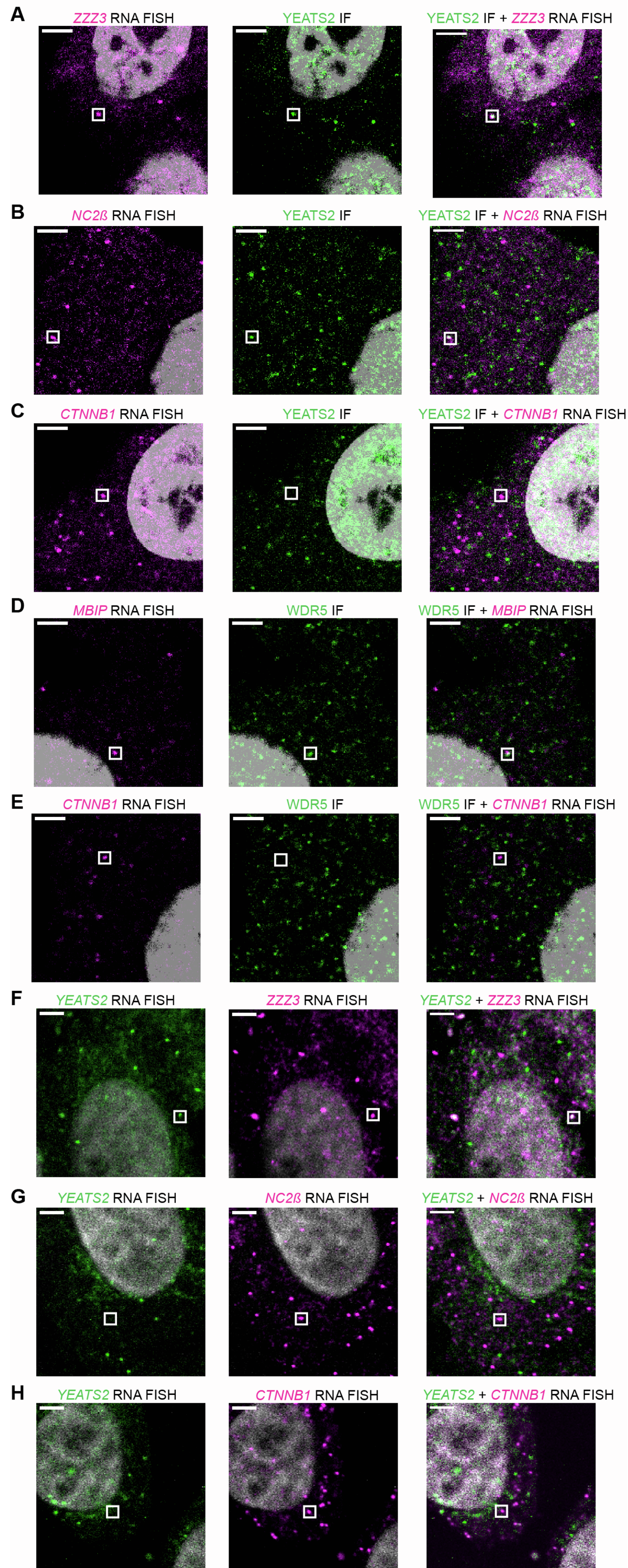
(right side) co-TA interactions are established either between two nascent protein partners or may occur by reciprocal sequential TA. (**B, C and E**) HeLa cells expressing N-terminally GFP tagged ATAC core subunits (in B and C) or SAGA core subunits (in E) were induced (+), or not (-) with DOX. In (B) whole cell extracts were made, separated on 6% (YEATS2 and ZZZ3) or 12% (WDR5) SDS PAGEs, and western blot analyses (WBs) were carried out with the indicated antibodies. In (C and E) polysome extracts were made and incubated with anti-GFP nanobody coupled beads, and bound proteins were separated on NuPAGE 4-12% Bis-Tris SDS PAGEs and analyzed by western blotting with an anti-GFP antibody. Arrows indicate the correctly expressed GFP fusion proteins. Molecular weight markers (M) are shown in kDa. (**D**) HeLa cells expressing N-terminally GFP tagged ZZZ3 (ATAC subunit) were either not-treated (NT), or treated with cycloheximide (CHX). Polysome extracts were prepared, anti-GFP RIPs carried out and analyzed by RT-qPCR as in Figure 2B (n=2).



Supplemental Figure 2. Co-localization of endogenous YEATS2 protein with its own mRNA and – or + CHX control experiments

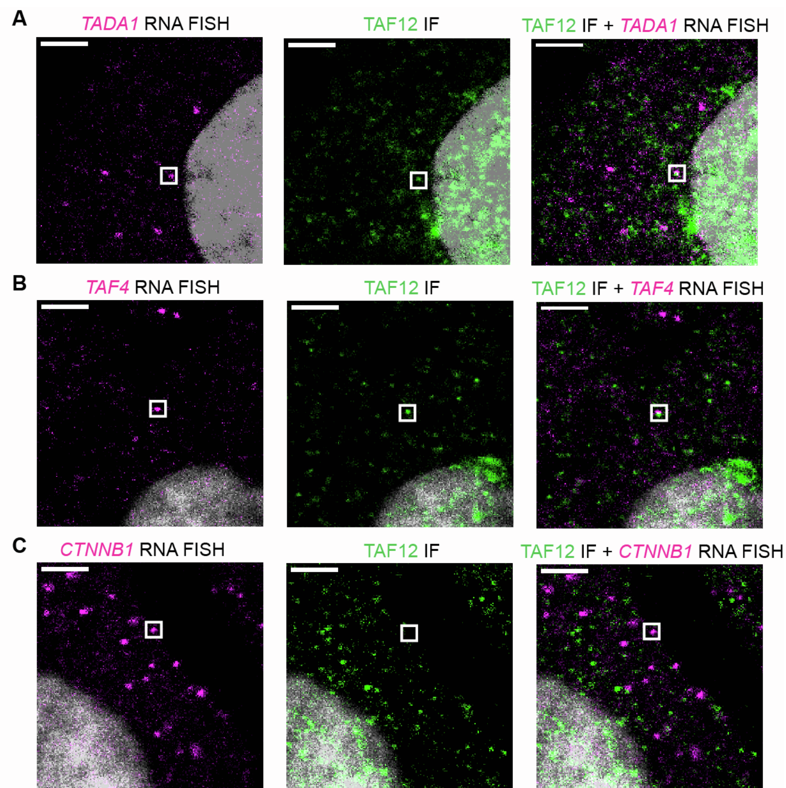
Cells were either non-treated (NT), treated with CHX or PURO (as indicated). (**A, B** and **C**) Confocal microscopy imaging was used to examine co-localization of endogenous YEATS2 protein with its own mRNAs by combining smiFISH and IF. (**A** and **B**) Representative multicolor confocal images for IF-coupled smiFISH images of fixed HeLa cells are shown. Each image is a single multichannel confocal optical slice. Co-localized spots are indicated with white rectangle and as zoom-in regions shown under every image. Scale bar (5 µm). Yellow arrowheads indicate colocalized spots. (**C** and **D)** Boxplots showing the percentage of

cytoplasmic RNA spots (as indicated at the bottom of the graphs) co-localizing with endogenous YEATS2 proteins in IF-smiFISH experiments. (**E**) Boxplots showing the percentage of cytoplasmic *YEATS2* RNA spots co-localized with the *ZZZ3* RNA target spots in dual-color smiFISH experiments using distinct secondary FLAP probes sequences. Each circle represents one biological replicate (N=3 in C; N=4 in D-E). For each condition, the number of cells analyzed is indicated in bracket above each boxplot. Unpaired two tailed t-tests were performed for statistical analyses between two different experimental condition (CHX and PURO). *p value ≤ 0.05, ***p value ≤ 0.001, ****p value ≤ 0.0001.



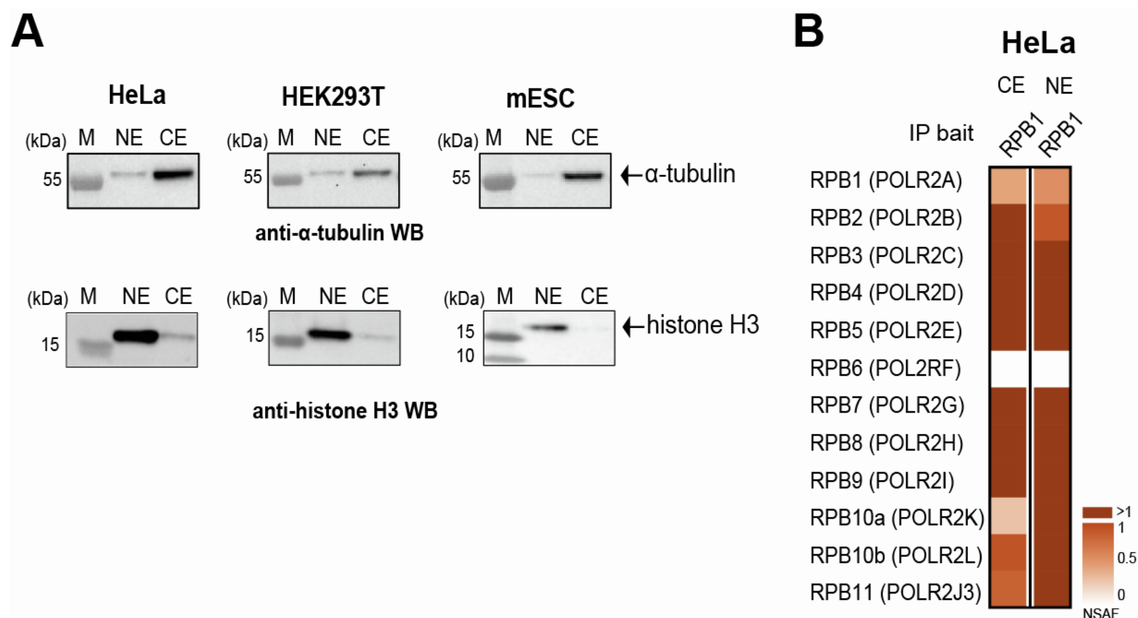
Supplemental Figure 3. Co-localization of endogenous ATAC subunits with mRNAs coding for their corresponding interacting partner, and mRNAs coding for simultaneous co-TA partners

Separate color panels are shown corresponding to Figure 3. In (**A-E**) smiFISH mRNA signals are shown in magenta; IF signals for YEATS2 or WDR5 proteins are in green. In (**G-H**) *YEATS2* smiFISH mRNA signal is in green, while *NC2β* or *CTNNB1* smiFISH mRNA signals are in magenta.



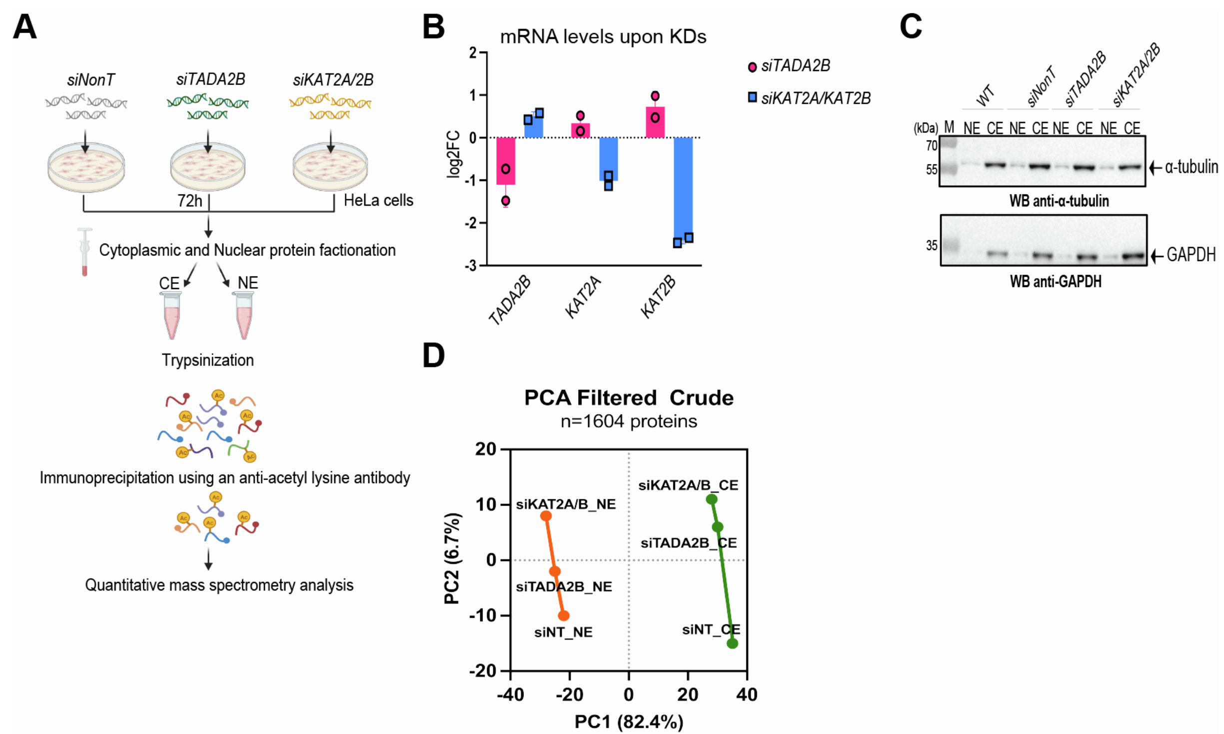
Supplemental Figure 4. Co-localization of endogenous SAGA subunits with mRNAs coding for their corresponding interacting partner.

Separate color panels are shown corresponding to Figure 4E-4H. In (**A-C**) smiFISH mRNA signals are shown in magenta; IF signals for proteins are in green.



Supplemental Figure 5. Control experiments verifying cellular fractionations prepared from different cell lines, and sub-cellular distribution of RNA polymerase II.

(**A**) Nuclear extracts (NEs) and cytoplasmic extracts (CEs) were prepared from human HeLa, human HEK293T and mES cells and the protein fractionation was tested by western blot analyses. Upper blots were developed with an anti-α-tubulin antibody and the lower blots with an anti-histone H3 antibody. Molecular weight markers (M) are shown in kDa. (**B**) Mass spectrometry analysis of anti-RPB1 IPs carried out using CE and NE (as indicated) prepared from HeLa cells. Three technical replicates were carried out (n=3). NSAF values were calculated.



Supplemental Figure 6. The SAGA complex acetylates non-histones proteins in the cytoplasm.

(**A**) Schematic representation of the workflow. (**B**) RT-qPCR analysis of mRNA levels upon *siTADA2B* and *siKAT2A/KAT2B* (*siKAT2A/2B)* knock-down. Error bars ± SD (N=2). *GAPDH* mRNA was used as an internal control. (**C**) Western blot analysis of separated NEs and CEs. The upper membrane was developed with an anti-α-tubulin antibody. The lower membrane was developed with an anti-GAPDH antibody. (**D**) Principal component analysis (PCA) of NEs and CEs.

Supplemental Tables

Supplemental Table 1. Oligonucleotide sequences for RT-qPCR

Gene name	**Forward primer**	**Reverse primer**
S100A11	CTGGTGTCCTTGACCGCAT	TTCTGGGAAGGGACAGCCTT
SNCA	ACCAAACAGGGTGTGGCAGAAG	TCATCATGCGGTCAAGGACAC
GFP	AGTCCGCCCTGAGCAAAGA	TCCAGCAGGACCATGTGATC
KAT2A	AGAGCTTTGGAGGCTTGGAT	TGAGCAGTTCTGGTCCTCAG
KAT2B	AGAGAGACAGGCTGGAAACC	GCTCTTGAGCGTGCTGTAAA
TADA3	AGCCCAAGAAGCAGAAACTG	ATTCCTGGATCTTGGGCTGAAG
SGF29	TGCAACATCCTTCGGAAAGC	TTGTAGAGACCGGCAATCTTGG
TADA2A	CGGGAGTCATCAAGCTTTGG	CCATGAGGTAGGAGGAGCAG
TADA2B	AAGAGTCGGCAGAGTACGAG	GTCTTTGCCGTCCTCCTTTC
YEATS2	TGGATGTTGAACTCCATCGC	AAAGATGGAGGGGCATCAGAG
ZZZ3	AGAAGGATGGAGAGTCCCTTTC	ATCATCTGAGGACGACTGCTTG
KAT14	AATGGATACCAGCCAGTCAGC	TTGCCATCTGCTGAGCAATC
MBIP	TTGGACAGCTTGACCTCAGAG	GTGCACTAAAGAGCAATGCAG
WDR5	AATTTGGGGCGCGTATGATG	AATCTGACGACCAGGCTACATC
NC2β	GGTGAACTGCTGCACTGAATTC	ATGACATGCTCTGGTGAGATGG
TAF5L	TGTGCCAACATAGTGTCTGC	AATTCCGCAGTCGTCCAAAC
TAF6L	CAGTGCTGTGTCTTCACTGAC	CGGATCATCCCCTAGCACAG
TAF9	ATCTCTTGGGGGAGGAGAGG	TGTTGGAGTTTGCCTTCCGA

TAF10	AGGCCGTGCCCTTCATTTTG	AGCTGCCCAGAAATTCATCTCA
TAF12	CTGAGACGAACGCTTCACTG	AACCTGGTCCTTCGAACACT
TADA1	GCCAGCTTGAAGGGAGAATG	GACAACAGCTGAAACAGCCT
SUPT3H	CAATGCCTGCTTCCCAACTT	GTATGGCATGTGCAGGAGTG
SUPT7L	ATCAGCAGCAGACAGAAGGT	TCATCAGGGAGAGGAGGTGA
SUPT20H	ATAGGGCAGCTGGAGAAAGC	CACCACAACAGACAAGCAGC
TRRAP	CAGCCCAGCAAATCATCGAA	TGTTCCTTCCCAGGTTGGTT
KPNB1	GATGACTGGAACCCCTGCAA	GTACCGCCAATCTGGGTTCT
KPNA2	CCTTAGTTCGGCTCCTGCAT	TGGGGCACAACTCCTGTTTT