# Supporting Information

## Cherrier et al. 10.1073/pnas.1220136110

### SI Materials and Methods

**Plasmids.** The constructs used in our assays have been described previously (1): pcDNA3, pshRNA-CTIP2, pshRNA-control, RFP-CTIP2, pFLAG-CTIP2, and pFLAG-CTIP2 deletion constructs.

**Coimmunoprecipitation Assays and Antibodies.** HEK293T cells cultured in 100-mm-diameter dishes were transfected using the calcium phosphate coprecipitation method with the indicated plasmids (30 μg). Two days posttransfection, immunoprecipitations were performed using the standard technique with M2 anti-FLAG (Sigma), anti-Hexim1 (Abcam), or anti-CyclinT1 (Santa Cruz) antibodies overnight at 4 °C with or without RNase or HMBA treatments. Finally, the immunoprecipitated complexes were processed for SDS/PAGE and Western blot analysis, real time quantitative RT-PCR (qRT-PCR), or RT-PCR assay. For real-time qRT-PCR experiments, 7SK snRNA was quantified for each immunoprecipitation, and results were normalized to the EEF1A1 copies. The presence of 7SK in immunoprecipitated complexes was also assessed by agarose gel electrophoresis after RT-PCR amplification. Proteins were detected in Western blot analyses using antibodies directed against the FLAG epitope (M2 mouse monoclonal from Sigma), CyclinT1 and Cdk9 (Santa Cruz), and CTIP2, Brd4, and HEXIM1 proteins (Abcam).

**Micro Scale Thermophoresis.** The Micro Scale Thermophoresis (MST) technique capitalizes on the fact that molecules move in a temperature gradient from hot to cold (thermophoresis). This movement is impacted by complex formation because molecules in a complex show an altered velocity. The MST technique allows one to follow the thermophoresis of a fluorescently labeled molecule (here Fam6-labeled RNA) as a function of increasing concentrations of a binding partner (here CTIP2). To obtain a series of successively decreasing CTIP2 concentrations, total cellular extracts from FLAG-CTIP2–expressing HEK293 cells were diluted with total cellular extract from nontransfected cells. For MST experiments, a series of 10 successive dilutions was used ranging from 1:0 (nondiluted extract) to 1:511. For binding, 5 μL of each dilution were incubated with 20 ng of Fam6-labeled 7SK L2 RNA or 7SK L2 m137 RNA for 10 min on ice in EMSA buffer (10 mM Hepes/KOH, pH 7.9, 100 mM KCl, 5 mM $MgCl_2$, 0.25 mM DTT, 0.1 mM EDTA, 10% glycerol, and 100-fold excess of yeast tRNA). MST experiments were conducted using a Monolith NT.115 (Nanotemper Technologies) as described previously (2) (Laser power 20%, Laser-on time 60 s, LED power 30%).

**Transcriptome Analyses.** For Fig. 4*A*, samples of 250 ng of total RNA from CTIP2 knockdown microglial cells and WT cells were amplified and labeled with Cy5 (e1a) and Cy3 (mock) (PerkinElmer), respectively, using the Agilent Two Colors Low RNA Input Linear Amplification Labeling Kit according to the manufacturer's instructions. Labeled RNA was hybridized to the Agilent Human whole-genome array (G2534-60011) and analyzed as recommended by the manufacturer. Expression data were extracted using Agilent Feature Extraction software (version 9.1.3.1). Raw data were natural Log (ln) transformed, and signals from multiple probes for the same gene were averaged. Data from three independent replicate experiments were averaged. The profiles were clustered using Spearman rank hierarchical clustering. For all other Fig. 4 microarray analyses, RNA amplification, labeling, hybridization, and detection were performed following the protocols supplied by Applied Biosystems using the corresponding kits (Applied Biosystems; product nos. 4339628 and 4336875). Data quality was determined using a QC procedure (3). Data were normalized using NeONORM with k = 0.02. Subtraction profiling was performed as in refs. 2 and 4 using the CDS test (5). In the case of cardiac hypertrophic mice, total RNA was purified from the cardiac ventricles with the RNeasy fibrous tissue kit (Qiagen). Total RNA quality was assessed on the basis of the A260/A280 ratio from the Agilent 2100 Bioanalyzer (Agilent Technologies). Microarray analysis for each group was performed on an Affymetrix GeneChip MOE 430 2.0 array (Affymetrix) on four selected RNA samples with the best quality score for each group in the Partnerchips platform (Evry, France) using the GeneChip scanner 3000 7G. The data were processed using GCOS 1.4 (GeneChip Operating Software). The data were analyzed as described previously (6-8).


1. Marban C, et al. (2007) Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. *EMBO J* 26(2):412–423.
2. Eilebrecht S, Bécavin C, Léger H, Benecke BJ, Benecke A (2011) HMGA1-dependent and independent 7SK RNA gene regulatory activity. *RNA Biol* 8(1):143–157.
3. Brysbaert G, Pellay FX, Noth S, Benecke A (2010) Quality assessment of transcriptome data using intrinsic statistical properties. *Genomics Proteomics Bioinformatics* 8(1): 57–71.
4. Eilebrecht S, et al. (2011) 7SK small nuclear RNA directly affects HMGA1 function in transcription regulation. *Nucleic Acids Res* 39(6):2057–2072.
5. Tchitchek N, et al. (2012) CDS: A fold-change based statistical test for concomitant identification of distinctness and similarity in gene expression analysis. *Genomics Proteomics Bioinformatics* 10(3):127–135.
6. Noth S, Brysbaert G, Pellay FX, Benecke A (2006) High-sensitivity transcriptome data structure and implications for analysis and biologic interpretation. *Genomics Proteomics Bioinformatics* 4(4):212–229.
7. Noth S, Brysbaert G, Benecke A (2006) Normalization using weighted negative second order exponential error functions (NeONORM) provides robustness against asymmetries in comparative transcriptome profiles and avoids false calls. *Genomics Proteomics Bioinformatics* 4(2):90–109.
8. Noth S, Benecke A; Systems Epigenomics Group (2005) Avoiding inconsistencies over time and tracking difficulties in Applied Biosystems AB1700/Panther probe-to-gene annotations. *BMC Bioinformatics* 6:307.

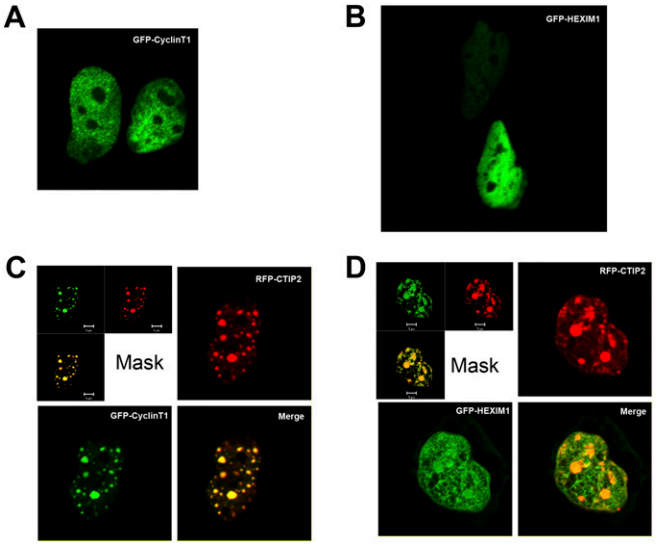


**Fig. S1.** CTIP2, CyclinT1, and HEXIM1 colocalize in nuclei. Cells were transfected with GFP-CyclinT1 (*A*) and GFP-Hexim1 (*B*) alone or in the presence of RFP-CTIP2 (*C* and *D*). Subnuclear locations were observed by confocal microscopy using Zeiss laser scanning microscope (Zeiss; model 510 inverted) equipped with a Planapo oil (63×) immersion lens (numerical aperture, 1.4).

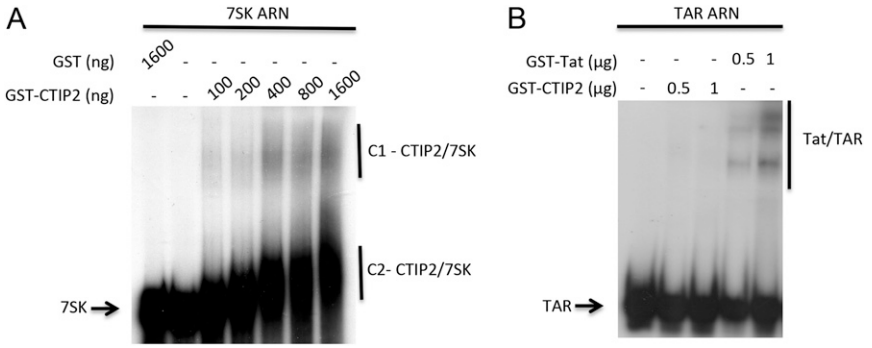


**Fig. S2.** CTIP2 binds the 7SK snRNA in vitro. Internally labeled 7SK RNA (*A*) or TAR RNA (*B*) and *Escherichia coli* total tRNA were renatured in binding buffer (30 mM Tris·HCl, pH 7.5, 300 mM NaCl, and 2.5 mM $MgCl_2$) and incubated with increasing concentrations of GST, GST-CTIP2, or GST-Tat. After incubation for 30 min at 37 °C and for 30 min at 4 °C in the presence of 0.01% Triton X-100, 5 μL of glycerol loading buffer was added, and the reaction mixture was loaded onto a native 5% polyacrylamide gel. Electrophoresis was performed in TB buffer at 150 V for 5 h at 4 °C.

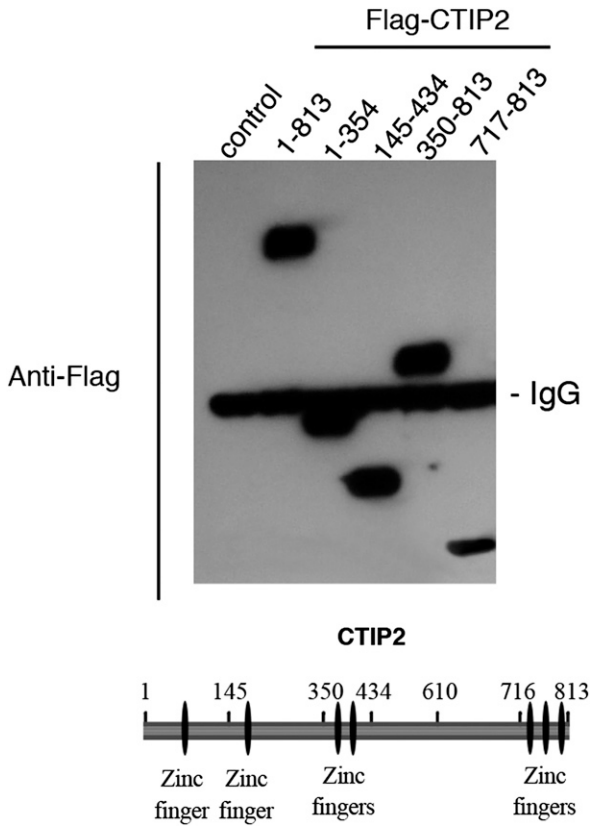


**Fig. S3.** Immunoprecipitations of truncated CTIP2 constructs. Flag-immunoprecipitated CTIP2 fragments used in Fig. 2D were visualized by Western blot analysis with anti-Flag antibodies.

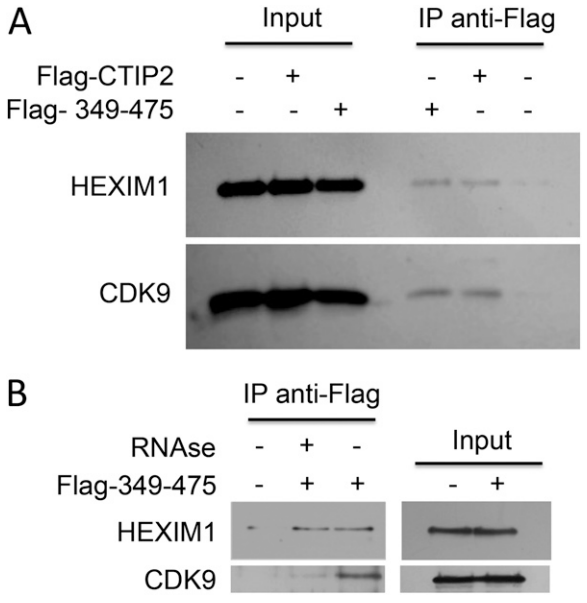


**Fig. S4.** (*A*) The 349–475 truncation of CTIP2 interacts with HEXIM1 and P-TEFb. Mock, Flag-CTIP2 wild-type or Flag-349–475 transfected HEK293T cells were lysed and subjected to immunoprecipitation with anti-Flag antibodies. Detection of immunoprecipitated proteins was determined by Western blot analysis with the indicated antibodies. (*B*) RNase treatment abrogates P-TEFb binding with the 349–475 truncation of CTIP2. Mock or Flag-349–475 transfected HEK293T cells were lysed and subjected to immunoprecipitation with anti-Flag antibodies in the presence or not of RNase A treatment as indicated. Detection of immunoprecipitated proteins was determined by Western blot analysis with the indicated antibodies.

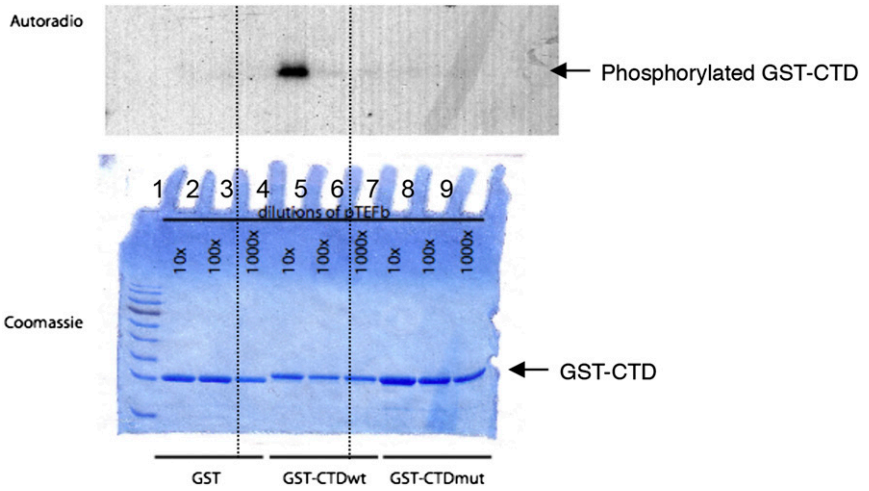


**Fig. S5.** Specific phosphorylations of the CTD Serin 2 and Serin 5 by purified pTEFb complexes. GST-, GST-CTD–, and S2/S5-mutated GST-CTD were subjected to phosphorylation assays with copurified CDK9/CyclinT1 (Milipore; ref. 14–685) (stock diluted 10× = 250 ng, 100× = 25 ng, and 1,000× = 2.5 ng of purified proteins per assay). GST proteins were separated by SDS/PAGE, and phosphorylations were revealed by autoradiography.

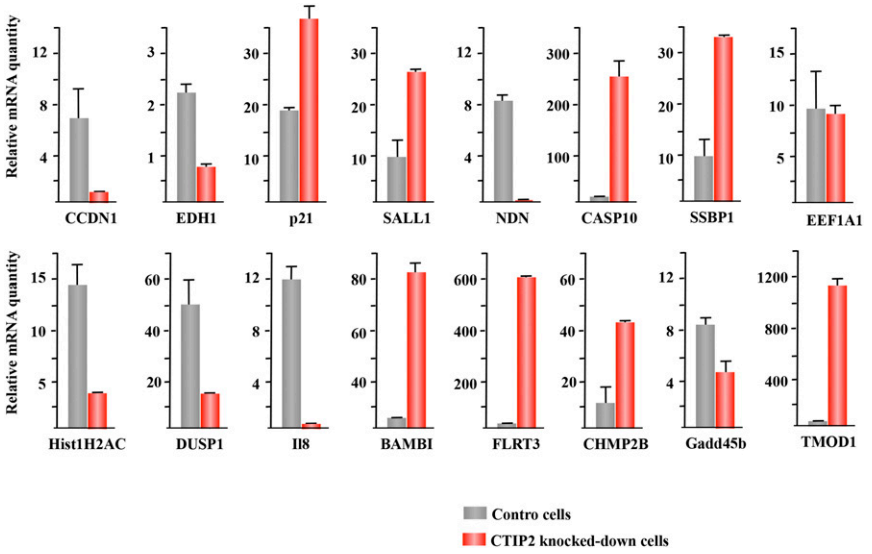


**Fig. S6.** Quantifications of CTIP2-sensitive genes in control and CTIP2-knocked down cells. Total RNA were extracted from control and CTIP2-knocked down cells to be subjected to RT-Q-PCR quantifications of the indicated mRNA. Results were presented relative to the housekeeping EEF1A1 gene.

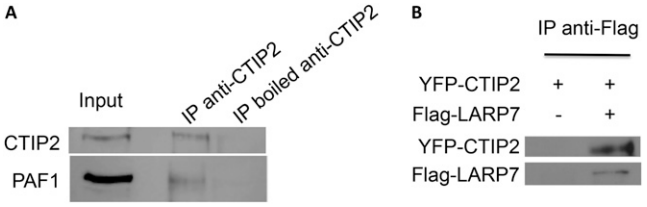


**Fig. S7.** CTIP2 Interacts with PAF1 and LARP7. (*A*) Nuclear extracts were subjected to immunoprecipitation experiments targeting the endogenous CTIP2. The presence of endogenous CTIP2 and PAF1 in the Ip was analyzed by Western blot. (*B*) Nuclear extracts from cells expressing Flag-LARP7 and YFP-CTIP2 were subjected to immunoprecipitation experiments targeting the Flag tag. The presence of YFP-CTIP2 and Flag-LARP7 proteins was analyzed by Western blot.

## Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)
Dataset S5 (XLS)
Dataset S6 (XLS)
Dataset S7 (XLS)