Supplementary Material and Methods

Cell culture

HeLa, HEK293, and NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) with 10% fetal bovine serum (FBS) with 100 U/ml penicillin-streptomycin and incubated at 37°C with 7% CO_2. CMK (purchased from DSMZ cell collection) and HL60 cells were cultured in RPMI-1640 (Gibco) with 20% fetal bovine serum and 100 U/mL penicillin-streptomycin and incubated at 37°C with 7% CO_2. Leucettine L41 was kindly provided by L Meijer [15,16].

Generation of inducible DYRK1A-expressing HEK293 cells

The cDNAs coding for either DYRK1A (WT) or the kinase-dead mutant of DYRK1A(K188R) were inserted into the pi-tk-hygro vector for retroviral delivery [17]. Two days after transfection using FuGENE (Promega E2311), the medium of the packaging HEK293 cells was filtered with a 0.45-µm filter (Millipore) and supplemented with polybrene (AL-118, Sigma) at 100 µg/ml. This medium was used to infect host HEK293 cells (three consecutive infections), followed by selection with hygromycin (H3274, Sigma) at a final concentration of 100 µg/ml.

Ethics Statement

Immunisation of rabbits was carried out by Eurogentec SA, Seraing. All animals used in the protocol were handled in strict accordance with good animal practices within the local jurisdiction (the Belgian national animal welfare regulations). Rabbit immunisation work at Eurogentec has approval from the ethics committee of the Centre d'Economie Rurale (CER Groupe, Marloie, Belgium).

Immunofluorescence and image acquisition

Transient transcription with a construct encoding GFP-DYRK1A-FLAG was performed using FuGENE HD (Promega E2311) reagent as recommended by the manufacturer. Immunofluorescent labeling was performed in NIH3T3 cells after transient transfection for 48 hours in culture. Cells were fixed with PBS-3.7% paraformaldehyde at room temperature (RT) for 10 min, then permeabilized with PBS-triton 0.5% at RT for 15 min, and incubated with primary antibodies at 4°C overnight. Next, coverslips were washed three times in PBS-BSA 0.5% (w/v) followed by 1h incubation with secondary antibodies at room temperature while protected from light. Then they were washed three times in PBS-BSA 0.5% (w/v), and once in PBS, before final staining with DAPI. Imaging was conducted on an Axiovert 200M microscope (Zeiss) coupled with an Apotome with Axiovision 4.7 (Zeiss).

In vitro *interaction experiments*

GST-HP1 fusions, GST-ΔBrg1 (1223–1420), and GST-DYRK1A constructs were described previously [18,19]. B10-tagged histone H3 was constructed in pET15b plasmids that also provided a 6x His affinity tag used for purification. Pull-down assays were performed in ELB buffer (HEPES pH 7, 250 mM NaCl, 1 mM EDTA, 0.1% NP40, 1x Complete protease inhibitor cocktail from Roche). Bound proteins were eluted in 100 mM Tris pH 8 with 20 mM glutathione, resolved by SDS-PAGE, and detected by western blotting using the B10 monoclonal mouse anti-estrogen receptor α antibodies (Euromedex ERB10-As) or anti-histone H3 rabbit polyclonal antibodies (ab1791 abcam).

In vitro *kinase assays*

GST or GST-DYRK1A were incubated in phosphorylation buffer (25 mM Hepes pH 6.8, 10 mM $MgCl_2$, 0.5 mM DTT, 200 µM ATT, 1x Complete protease inhibitor cocktail from Roche, 1% BSA) with rH3(25–66) and rH3 (Biolabs M2507S) in a total volume of 15 µl for 30 minutes with shaking at room temperature.

Depletion of DYRK1A by siRNA

The following siRNA were synthesized by Dharmacon (ON-TARGET plus): siDYRK1A-05 (J-004805-05), siDYRK1A-06 (J-004805-06), non-targeted siNT (D-001810-01), siHP1α (5-CACAAAUUGUGAUAGCAUU-3), siHP1β (5-AGCUCAUGUUCCUGAUGAA-3), and siHP1γ (5-AUCUGACAGUGAAUCUGAU-3). siRNA were transfected into HeLa cells at a final concentration of 50 nM using DharmaFECT1 (T-2001). Cells were harvested three days after transfection. RNA was extracted using the High Pure RNA Isolation Kit (Roche Ref: 11 828 665 001). Levels of mRNA were quantified by real-time PCR after reverse transcription with 200u of M-MLV reverse transcriptase according to the manufacturer's recommendations (Invitrogen). Proteins were extracted with 8 M urea and detected by western blotting using anti-DYRK1A (Sigma ref: WH0001859M1), anti-HP1α (2G9), HP1β (1A9), and HP1γ (1G6) from Euromedex.

Human exon array data analysis

Microarray dataset analysis and visualization were made using EASANA® (GenoSplice technology), which is based on the GenoSplice's FAST DB® annotations [18]. For this analysis, we conducted an unpaired Student *t* test to compare gene intensities between HeLa cells transfected with non-targeted siRNA and HeLa cells transfected with an

siRNA targeting DYRK1A mRNA. Genes were considered significantly differentially expressed when fold change was ≥1.5 and $P \leq 0.05$ (unadjusted P). Gene Ontology analysis of genes affected by depletion of DYRK1A was performed using PANTHER tools (http://www.pantherdb.org and [21]). Genes regulated by both DYRK1A and NFκ-B and listed in Table S2 were selected by crossing the hits from the DYRK1A depletion arrays with data from an on-line resource providing a list of NFκ-B genes (http://bioinfo.lifl.fr/NF-KB/).

Chromatin immunoprecipitation (ChIP)

ChIP was performed essentially as previously described [22], with minor alterations. HeLa cells were cross-linked in phosphate-buffered saline (PBS) containing 1% formaldehyde (Sigma) for 10 min at room temperature. The crosslinking reaction was quenched with PBS containing 125 mM glycine, followed by three washes with ice-cold PBS. The chromatin was fragmented by sonication, to produce average DNA lengths of 0.5 kb. Then, chromatin was immunoprecipitated with anti-histone-modification antibodies (ab1791, ab8898, ab26127, H3pS57, 1H5, ab40828, 42s2, 2E12, and WH0001859M1). After ChIP, the eluted DNAs were detected by qPCR using the primers listed in Table S3. Levels of histone modification are expressed as % of H3, and levels of other proteins are expressed relative to the signal obtained for ChIP using non-immune IgGs. Values are averaged from three independent experiments.

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