Supplemental Materials

Molecular Biology of the Cell

Hajingabo et al.

Supplemental figure 1. *ETV6-RUNX1* is recruited to the *CDKN1A* promoter. A. Schematic representation of ETV6 (TEL) and RUNX1 (AML1) proteins, and the fusion protein ETV6-RUNX1. Adapted from (Zelent et al. 2004) and UNIPROT entries P41212 and Q01196. **B**. ETV6-RUNX1 (E/R) knockdown in REH cells was performed by shRNA lentiviral transduction using the plasmid pLenti6/BLOCK-iT^{TM}-DEST-shG1 and a non-targeting control vector expressing shRNA against LacZ (pLenti6/BLOCK-iT^{TM}-DEST-shC) to generate REH-G1 and REH-C, respectively. Cells were selected for shRNA expression using blasticidin (5µg/ml) and knockdown of E/R monitored by Taqman RT-qPCR cells with at least 50% reduction were used in ChIP experiments. **C.** 10^7 REH-G1, REH-C cells were collected, DNA-protein cross-linked using formaldehyde and lysed using Diagenode lysis buffer iL1 and iL2. Chromatin DNA was sheared by sonication and ChIPs performed using anti-ETV6 (Santa Cruz N-19) or normal IgG as negative control, as indicated. 1% of input chromatin DNA was used as positive control. DNA was purified, quantified and an aliquot was used for PCR amplification using specific *CDKN1A* specific primers. PCR products were analyzed by agarose gel electrophoresis. **D.** Repression of *CDKN1A* promoter by ETV6-RUNX1. HEK293 cells were transfected with the reporter plasmid pCDKN1A-luc and indicated effector plasmids (pFlag-ETV6-RUNX1, pMX-MYC). Cells were treated with 100 µg/ml of PMA 12 hours post-transfection. Firefly luciferase data were normalized to renilla luciferase counts, and data are reported as mean and standard error of 3 independent experiments in triplicate.

Supplemental figure 2. Involvment of *TCF3-PBX1* in RNA transport A. Colocalization of NXF1 and TCF3-PBX1. HeLa cells were transfected with 1µg of pYFP-NXF1 and/or pFlag-TCF3-PBX1 plasmids using lipofectamine. Twenty-four hours post-transfection, cells were fixed in formaldehyde, permeabilized, incubated with anti-flag M2 antibody followed by Alexa 568 secondary antibody (Red). Cells were mounted with the Prolong gold Antifade reagent with DAPI (Blue) and the slides were analyzed by confocal microscopy using the Nikon A1R system and images processed with the IMARIS software. **B.** expression of *TCF3-PBX1* affects RNA localization. HeLa cells were transfected with 1µg of pFlag-TCF3-PBX1 plasmid using lipofectamine. Twenty-four hours post-transfection, cells were fixed in formaldehyde, permeabilized, incubated with anti-flag M2 antibody followed by Alexa 568 secondary antibody (Red). Cells were stained with the SYTO RNA marker (Green) and mounted with the Prolong gold Antifade reagent with DAPI DNA marker (Blue) and the slides were analyzed by confocal microscopy using the Nikon A1R system and images processed with the IMARIS software.

Supplemental table 1
Perturbed interactions in ETV6-RUNX1 samples
Supplemental table 2
Perturbed interctions in TCF3-PBX1 samples
Supplemental table 3
Perturbed interctions in BCR-ABL1 samples
Supplemental table 4
Perturbed interctions confirmed by ChIP-seq

Supplemental table 5

Relative expression of cell cycle genes interacting with MYC

Supplemental table 6

Deregulated pathways in ETV6-RUNX1 samples

Supplemental table 7

Deregulated pathways in TCF3-PBX1 samples

Supplemental table 8

Perturbed interactions in TCF3-PBX1 involved in RNA processing

Supplemental table 9

Similar target genes for MYC and ETV6-RUNX1

Figure 1

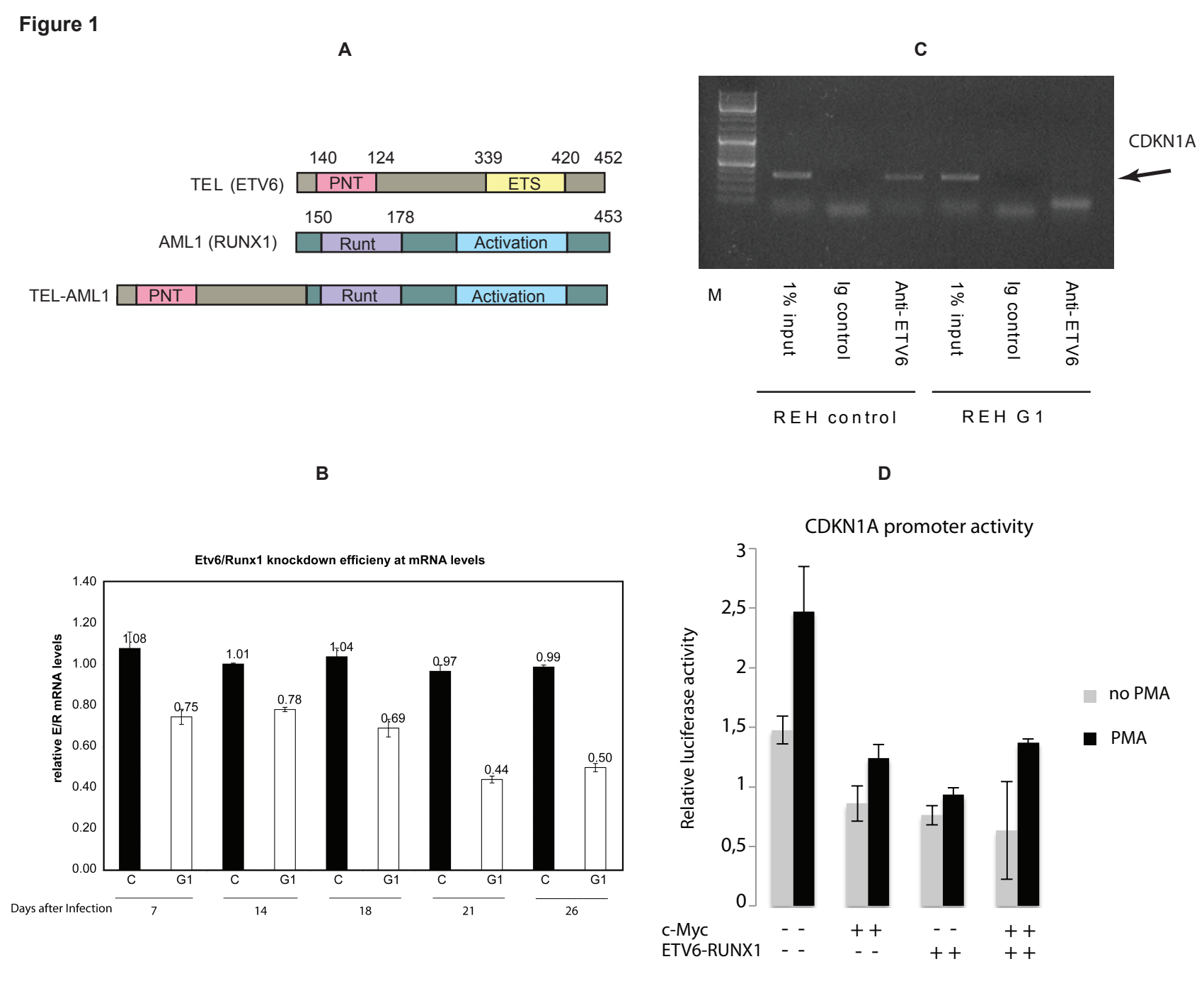
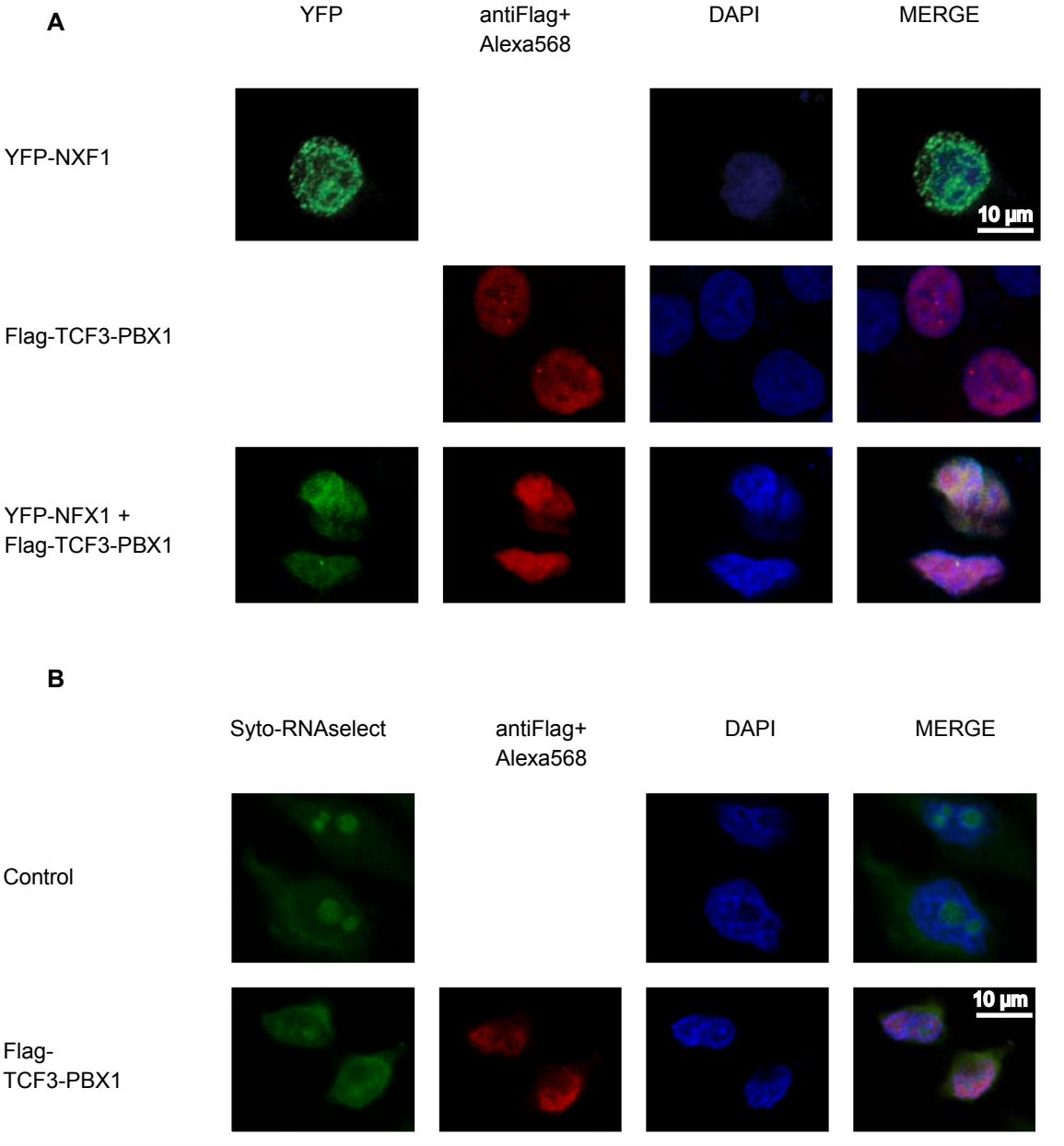

A
140
124
339
420
452
TEL (ETV6)
PNT
ETS
150
178
453
AML1 (RUNX1)
Runt
Activation
TEL-AML1
PNT
Runt
Activation
C
CDKN1A
M
1% input
Ig control
Anti-ETV6
1% input
Ig control
Anti-ETV6
REH control
REH G1
B
Etv6/Runx1 knockdown efficieny at mRNA levels
relative E/R mRNA levels
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.08
0.75
1.01
0.78
1.04
0.69
0.97
0.44
0.99
0.50
C
G1
C
G1
C
G1
C
G1
C
G1
Days after Infection
7
14
18
21
26
D
CDKN1A promoter activity
Relative luciferase activity
3
2,5
2
1,5
1
0,5
0
no PMA
PMA
c-Myc
ETV6-RUNX1
- -
- -
+ +
- -
- -
+ +
+ +
+ +

Figure 2


A
YFP
antiFlag+
Alexa568
DAPI
MERGE
YFP-NXF1
10 µm
Flag-TCF3-PBX1
YFP-NFX1 +
Flag-TCF3-PBX1
B
Syto-RNAselect
antiFlag+
Alexa568
DAPI
MERGE
Control
10 µm
Flag-
TCF3-PBX1