SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

Figure S1, related to Figure 2. CDK11 increases levels of expression from a variety of promoters in Jurkat cells. Plasmid targets containing LTR, CMV, cJun, MCK (muscle creatine kinase), EIAV (equine infectious anemia virus) and RSV (Rous sarcoma virus) promoters linked to the luciferase reporter gene were co-expressed with f:CDK11. Presented are their increased luciferase activities in the presence of f:CDK11 over the empty plasmid vector control (bar 1). Error bars represent mean ± SE of 3 independent experiments. Western blots of CDK11 and actin are presented below the bar graph.

Figure S2, related to Figure 2. Functional validation of the CDK11 proteome in 293T cells.

A. THOC1 inhibits increased HIV gene expression by f:CDK11. Presented is the luciferase activity of siScr RNA control and THOC1-depleted HeLaP4 cells co-expressing plasmid vector, f:CDK11 and pNL4-3Luc. Error bars represent mean ± SE of 3 independent experiments. Western blots of THOC1 and CDK11 with actin used as a loading control are presented below the bar graph.

B. THOC5 inhibits increased HIV gene expression by f:CDK11. Luciferase activity of siScr RNA control and THOC5-depleted HeLaP4 cells co-expressing f:CDK11 and pNL4-3.Luc. Error bars and western blots are as in panel A.

C. eIF4A3 does not affect increased HIV gene expression by f:CDK11. Luciferase activity in siScr RNA control and eIF4A3-depleted cells co-expressing vector, f:CDK11 and pNL4-3.Luc. Error bars and western blots are as in panel A.

D. CK2 does not affect increased HIV gene expression by f:CDK11. SiScr RNA control or CK2-depleted HelaP4 cells co-expressed vector, f:CDK11 and pNL4-3.Luc. Error bars and western blots are as in panel A.

Figure S3, related to Figure 3. CDK11 depletion affects HIV mRNA splicing also in Jurkat cells. CDK11 depleted Jurkat cells were co-expressed with the same plasmids as in Figure 3B. Presented are RT-qPCR data with primers depicted in Figure 3A. Error bars represent mean ± SE of 3 independent experiments. Western blots of CDK11 and actin are presented below the bar graph.

Figure S4, related to Figure 5. Knockdown of THOC1 has similar effects to those of CDK11 depletion. A, B, C, D, E, F. Effects of THOC1 depletion (A, B, C, D, E) and CDK11 depletion (F) on levels of RNAPII, Ser2P, Ser5P, CDK11 and CstF77 at the HIV gene.

Figure S5, related to Figure 6. CDK11 depletion increases read through transcription of a dual fluorescence plasmid reporter.

A. P_{CMV} plasmid contains the CMV promoter linked to RFP and EGFP reporter genes (Ji et al., 2011). PolyA sites were placed at the 3' end of each reporter gene. EGFP is also translated via an IRES placed at its 5' end. If 3' end formation occurs after RFP transcription, cells turn yellow. If the polyA site is ignored, EGFP is also expressed and cells turn yellow. Quadrants represented in this panel guide the analysis of FACS in panel B.

B. Presented is the two-dimensional FACS of 293T cells co-expressing siScr and $P_{CMV.}$

C. Presented is the two-dimensional FACS of CDK11-depleted 293T cells expressing P_{CMV}.

D. Presented is the two-dimensional FACS of CDK12-depleted 293T cells expressing P_{CMV}.

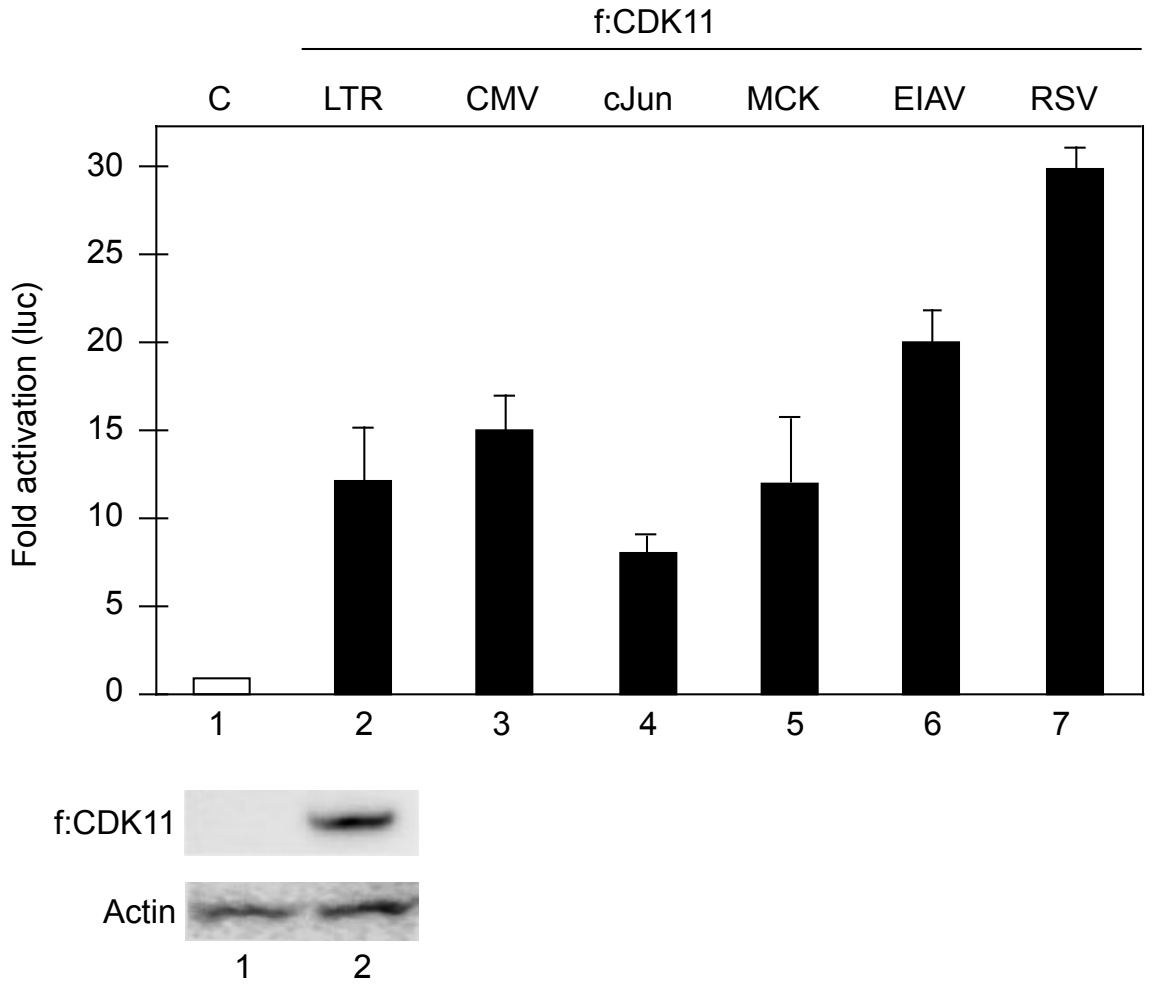
SUPPLEMENTAL METHODS

Dual fluorescence reporter system

293T cells were inoculated with siScr, siCDK11 or siCDK12 RNAs for 24 hours and then transfected with 2 μg of P_{CMV} (Ji et al., 2011). After an additional 24 hours cells were fixed in 2% paraformaldehyde and their red and green fluorescence was measured at 585 nm and 530 nm, respectively, on a BD FACSCalibur system (BD Biosciences). Analysis of FACS data was performed using FlowJo v10.0.7 (Tree Star Inc.).

REFERENCE

Ji, Z., Luo, W., Li, W., Hoque, M., Pan, Z., Zhao, Y., and Tian, B. (2011). Transcriptional activity regulates alternative cleavage and polyadenylation. Mol. Syst. Biol. *7*, 534.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LTR
CMV
cJun
MCK
EIAV
RSV
Fold activation (luc)
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25
20
15
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5
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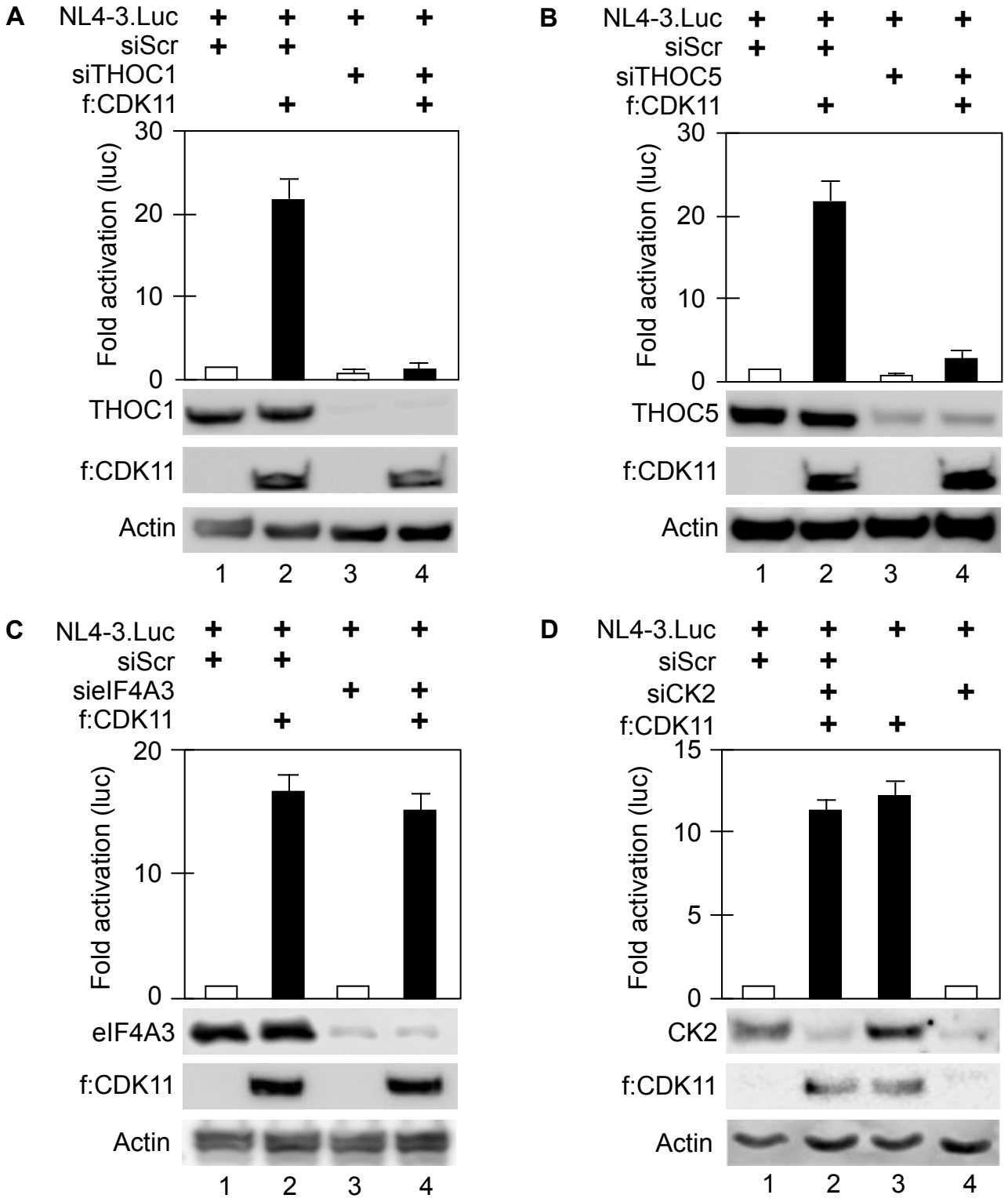
1
2
3
4
5
6
7
f:CDK11
Actin
1
2

Fig.S1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siTHOC1 + +
f:CDK11 + +
Fold activation (luc)
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20
10
0
THOC1
f:CDK11
Actin
1 2 3 4
B
NL4-3.Luc + + + +
siScr + +
siTHOC5 + +
f:CDK11 + +
Fold activation (luc)
30
20
10
0
THOC5
f:CDK11
Actin
1 2 3 4
C
NL4-3.Luc + + + +
siScr + +
sieIF4A3 + +
f:CDK11 + +
Fold activation (luc)
20
10
0
eIF4A3
f:CDK11
Actin
1 2 3 4
D
NL4-3.Luc + + + +
siScr + +
siCK2 + +
f:CDK11 + +
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10
5
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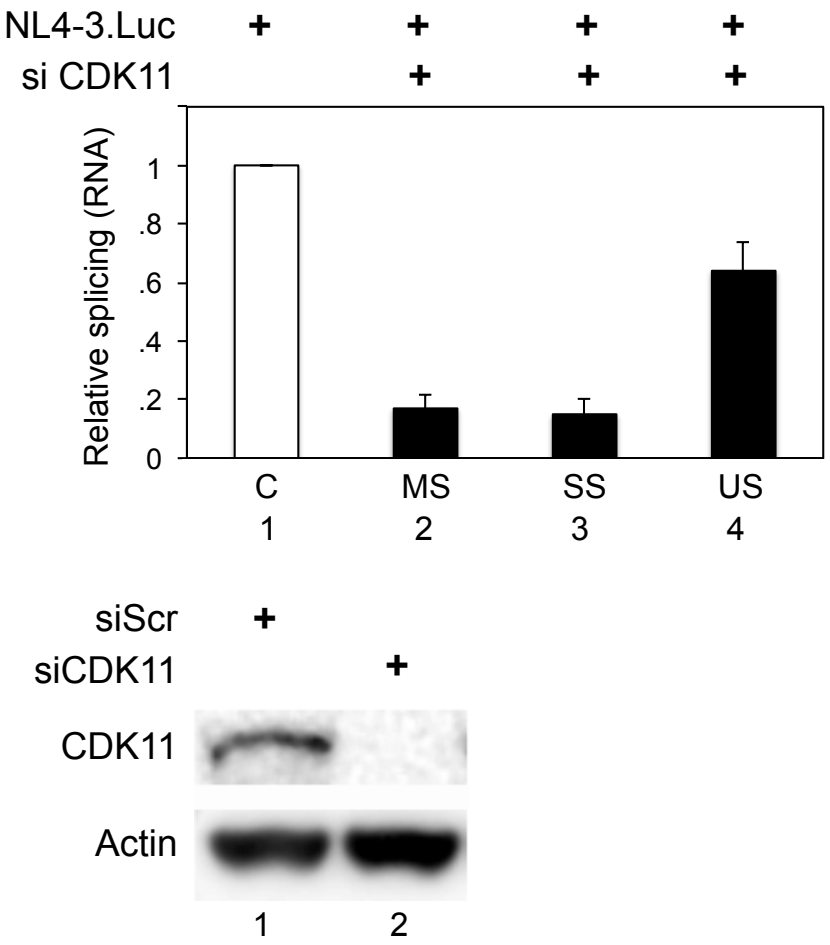
CK2
f:CDK11
Actin
1 2 3 4

Fig.S2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si CDK11 + + +
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.6
.4
.2
0
C
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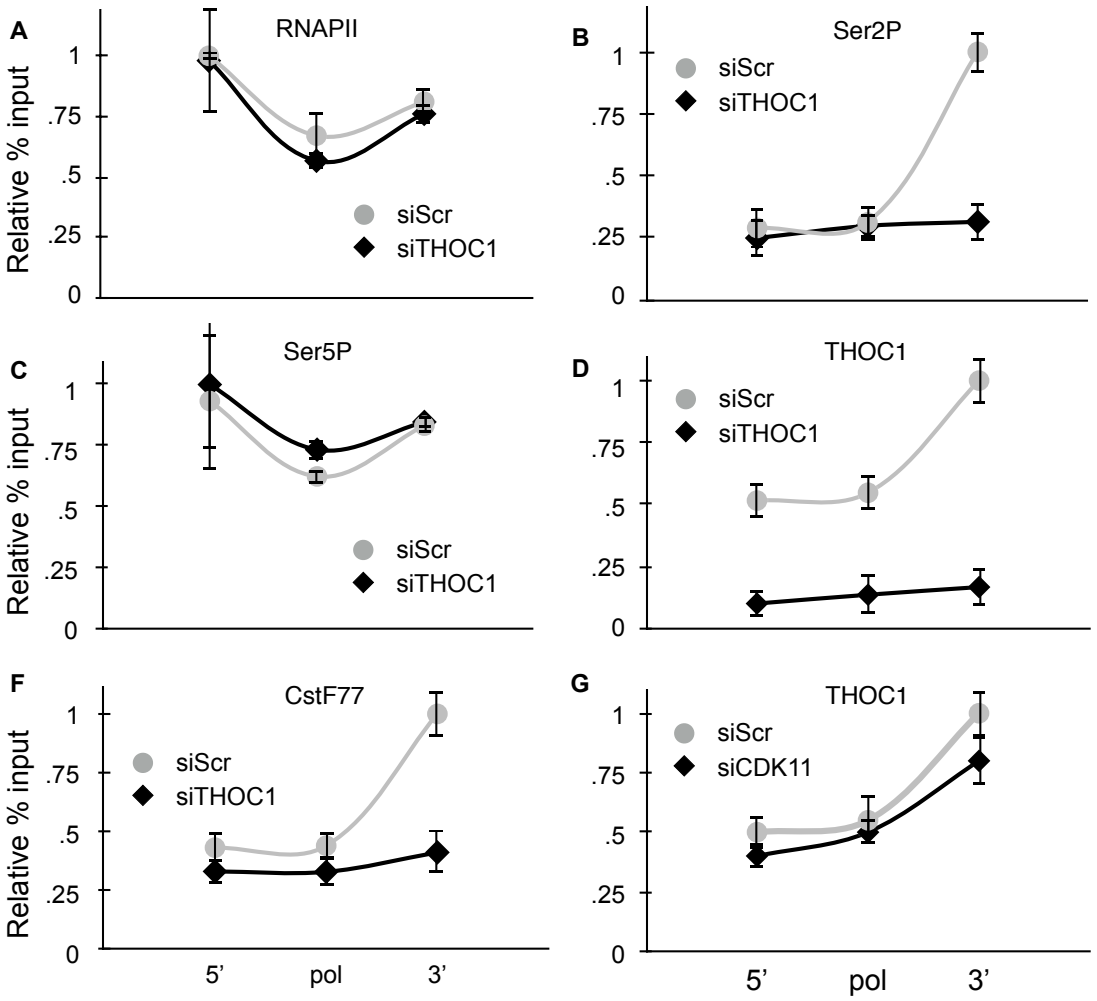
SS
US
1
2
3
4
siScr +
siCDK11 +
CDK11
Actin
1
2

Fig.S3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.25
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siTHOC1
B
Ser2P
siScr
siTHOC1
C
Ser5P
Relative % input
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siTHOC1
D
THOC1
siScr
siTHOC1
F
CstF77
Relative % input
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pol
3’
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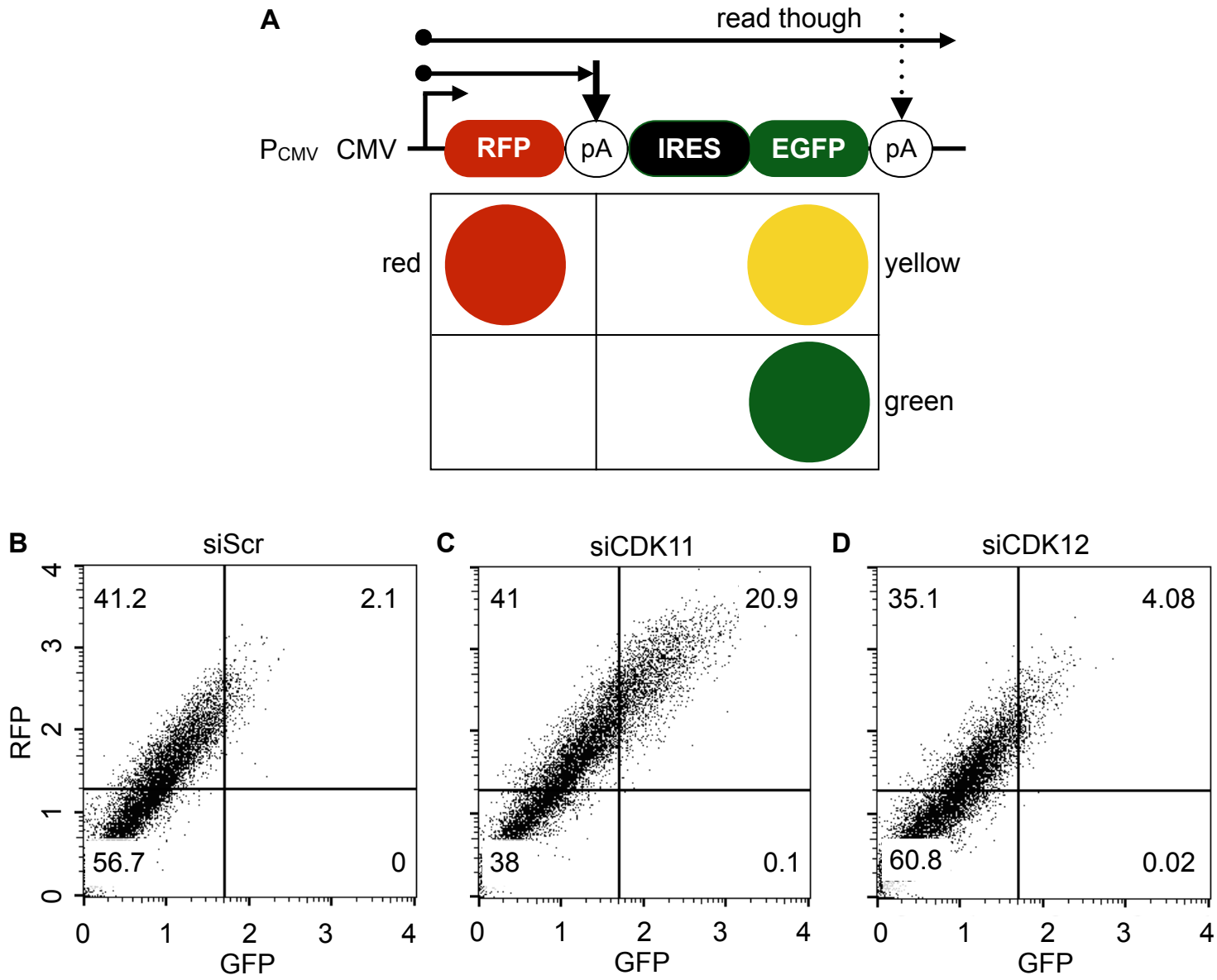
5’
pol
3’

Fig.S4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CMV
RFP
pA
IRES
EGFP
pA
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yellow
green
B
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0
C
siCDK11
41
20.9
38
0.1
D
siCDK12
35.1
4.08
60.8
0.02
RFP
GFP
0
1
2
3
4

Fig.S5