Supplemental Figures

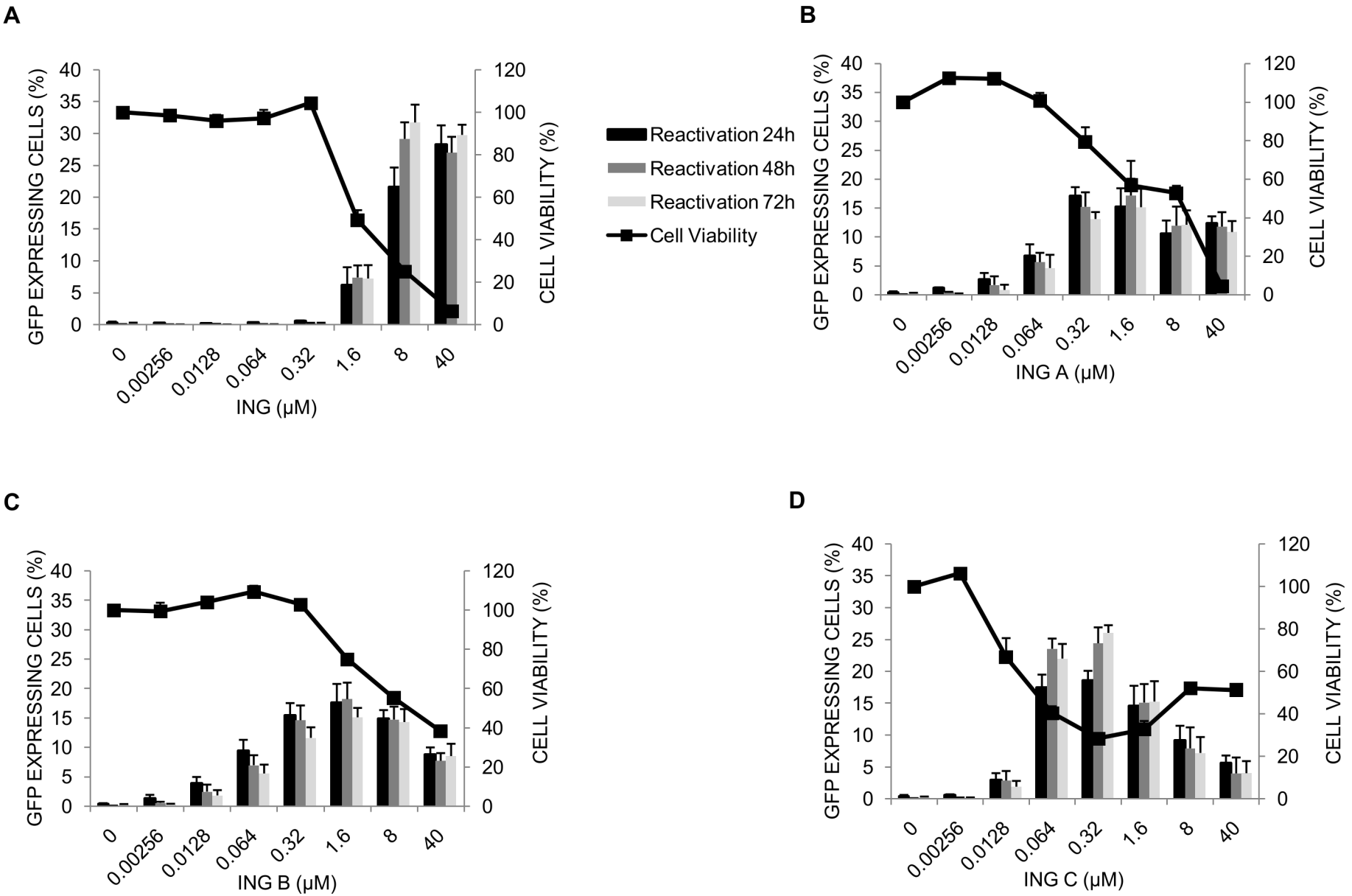
Fig. S1: Screening of ingenol derivates to activate HIV transcription. Ingenol esters were purified from *E. tirucalli* latex. Three different molecules were obtained with modifications at the C-3 position (ING A, B and C). J-Lat 6.3 was exposed to increasing concentrations of those compounds and the GFP expression evaluated 24 h (black columns), 48 h (dark gray columns) and 72 h (light gray columns) post-treatment. Cytotoxic effects were also evaluated and the cell viability plotted in the graphics as a black line. Cell viability *versus* reactivation levels are presented to: (**A**) the parent ingenol (ING), (**B**) ING A (ingenol 3-trans-cinnamate), (**C**) ING B (3-caproyl-ingenol), (**D**) ING C (ingenol 3-dodecanote).

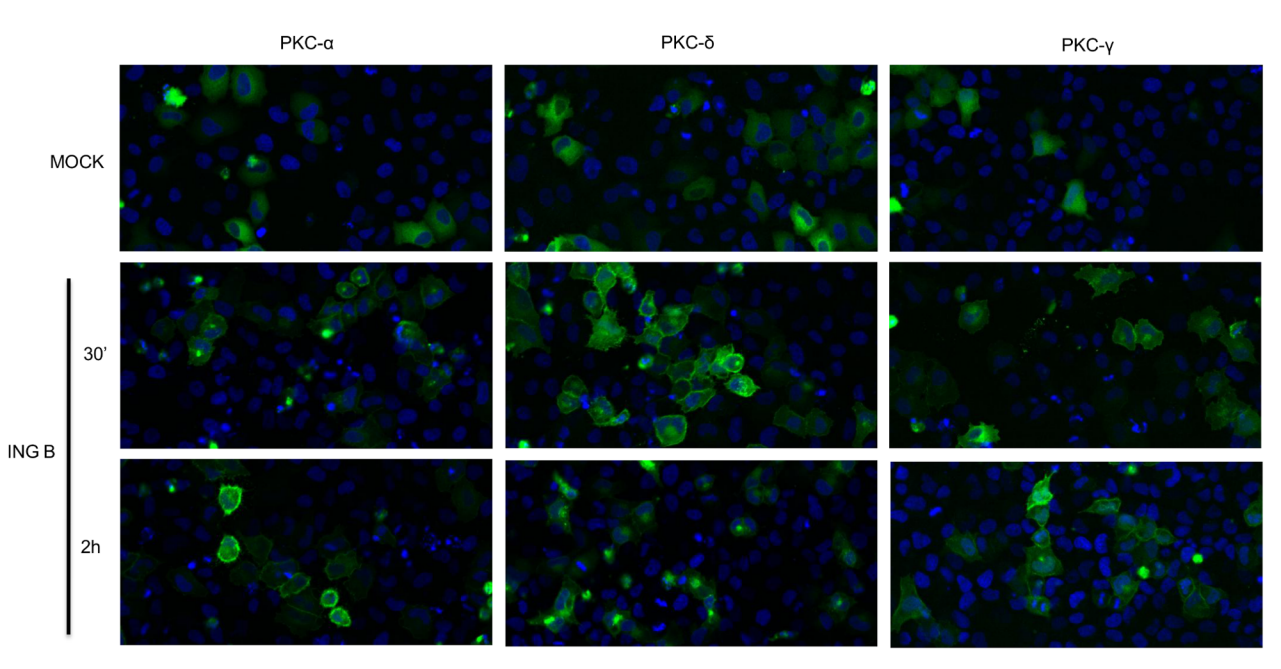
Fig. S2: PKC activation by ING B is maintained for longer periods. HeLa cells expressing PKC-GFP fused proteins were submitted to ING B (0.32 μM (0.32 μm = 0.00126 mil)) treatment at longer incubation times (30 min and 2 h). Different PKC-GFP isoforms are presented: PKC-α (left columns), PKC-δ (central columns) and PKC-γ (right columns). Cell nuclei are labeled with DAPI (blue color) and PKC-GFP are labeled in green. All the cells were analyzed by confocal microscope. Non-treated cells are presented in the first lane panels (mock) showing the diffuse pattern of PKC-GFP proteins.

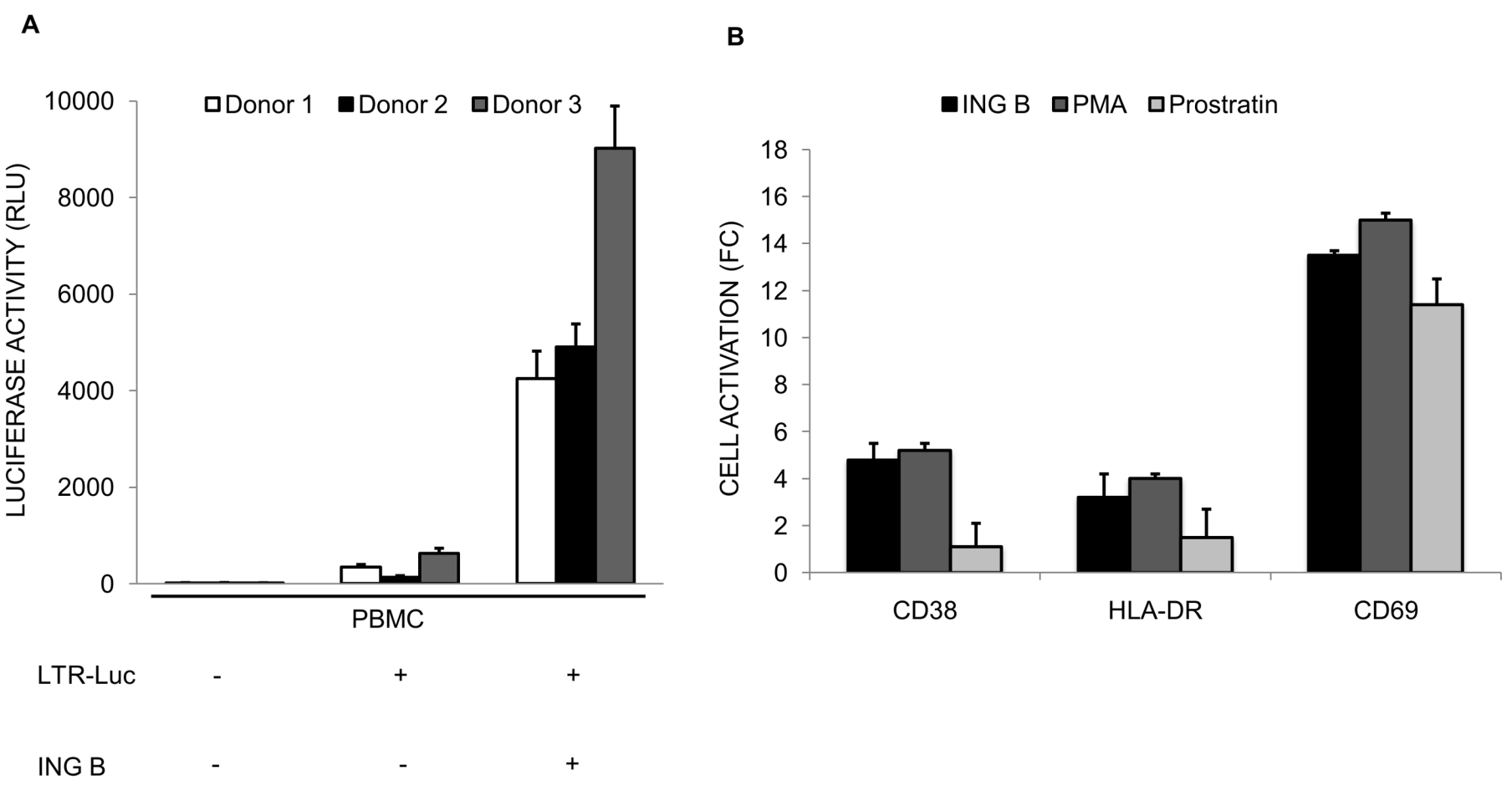
Fig. S3: ING B promotes transcription of luciferase reporter gene controlled by HIV-1 LTR and cellular activation in primary cells. (**A**) PBMCs were isolated from three different healthy donors designated here by Donor 1 (white columns), Donor 2 (black columns) and Donor 3 (gray columns). PBMCs were transfected with *pBlue3'LTR-Luc* (LTR-Luc) and treated with ING B (1 μM (1 μm = 0.00394 mil)). Luciferase reporter gene activity was measured 24 h post ING B treatment. Here we present the standard errors of three independent experiments. (**B**) Different activation markers (CD38, HLA-DR and CD69) were analyzed in T CD4+ cells from three HIV negative blood donors by flow cytometer. Cells were treated with 1 μM (1 μm = 0.00394 mil) of ING B, PMA and prostratin for 48 h. The results are presented as fold change related to non treated cells.

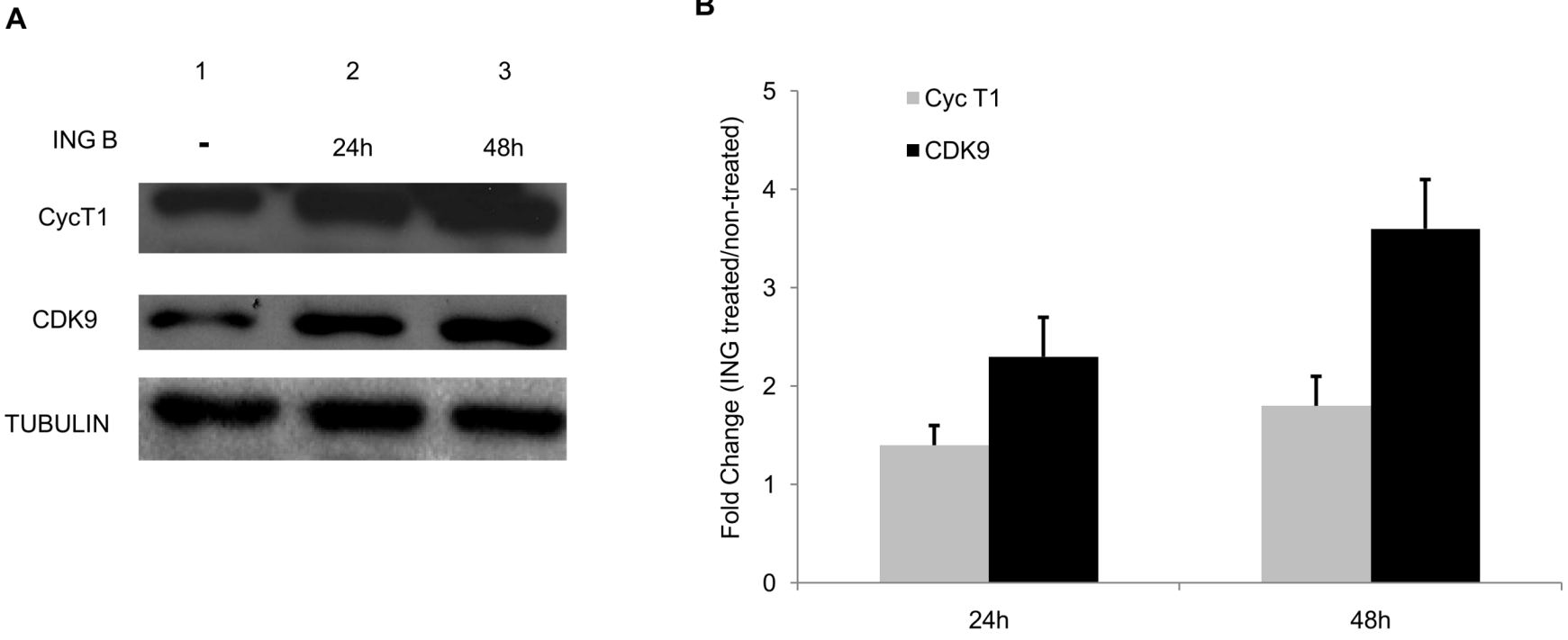
Fig. S4: ING B induces higher levels of P-TEFb components in latent cells. (**A**) J-Lat 6.3 cells were treated with ING B (0.32 μM (0.32 μm = 0.00126 mil)) for 24 or 48 h and the levels of Cyc T1 and CDK9 were detected by immunoblotting. Tubulin was used as loading control. Non-treated cells are presented in line 1. (**B**) Quantification of the immunoblots is shown as fold change (treated/non-treated cells ratio). Error bars indicate the standard deviation of triplicate experiments.

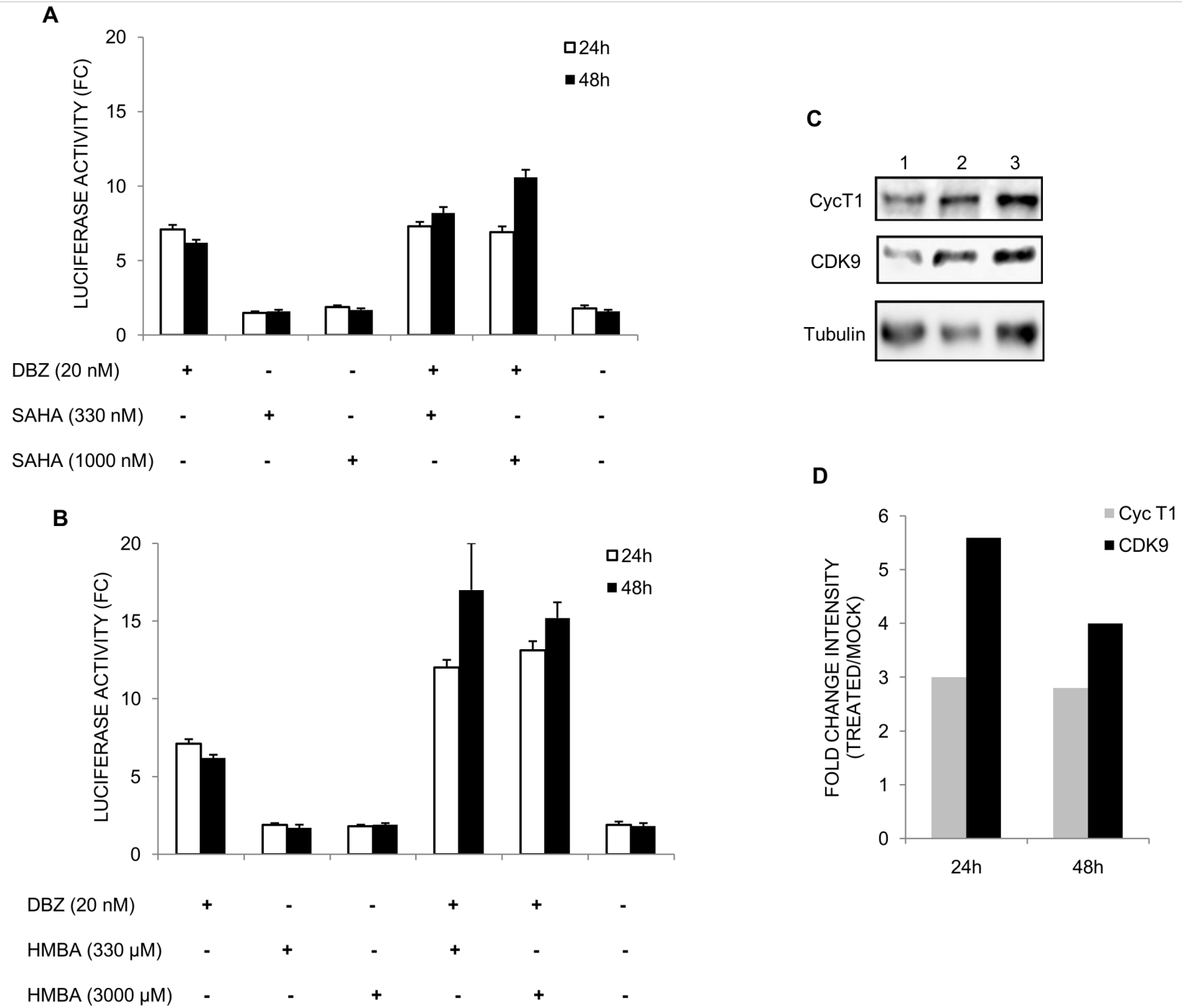
Fig. S5: Ingenolic compounds are able to reactivate HIV-1 latency in primary cells. Ingenol 3,20-dibenzoate was used to confirm the results obtained with ING B in latently infected primary resting CD4+ T cells. HIV-1 latency reactivation was measured by luciferase activity 24 and 48 h after induction (white and black bars respectively). (**A**) Ingenol 3,20-dibenzoate (20 nM (20 Nm = 14.8 ft-lb)) was used alone or in combination with SAHA (330 and 1000 nM (1,000 Nm = 738 ft-lb)). Error bars indicate the standard deviation of triplicate experiments. (**B**) Ingenol 3,20-dibenzoate (20 nM (20 Nm = 14.8 ft-lb)) was used alone or in combination with HMBA (330 and 3000 μM (3,000 μm = 0'0-4/32")) to latency reactivation in primary CD4+ T cells. Error bars indicate the standard deviation of triplicate experiments. (**C**) Levels of P-TEFb components, Cyc T1 and CDK9, were determined by immunoblotting 24 h (lane 2) and 48 h (lane 3) after ingenol 3,20-dibenzoate treatment (20 nM (20 Nm = 14.8 ft-lb)). Lane 1 represents non-treated cells. Tubulin was used as loading control. (**D**) Densitometry of CDk9 and Cyclin T1 bands are shown as fold change (treated/non-treated cells ratio). Cyc T1 and CDK9 are represented by gray and black bars, respectively.


A
B
C
D
GFP EXPRESSING CELLS (%)
CELL VIABILITY (%)
Reactivation 24h
Reactivation 48h
Reactivation 72h
Cell Viability
0
0.00256
0.0128
0.064
0.32
1.6
8
40
ING (μM)
ING A (μM)
ING B (μM)
ING C (μM)


PKC-α
PKC-δ
PKC-γ
MOCK
ING B
30'
2h


A
Donor 1
Donor 2
Donor 3
LUCIFERASE ACTIVITY (RLU)
10000
8000
6000
4000
2000
0
PBMC
LTR-Luc - + +
ING B - - +
B
ING B
PMA
Prostratin
CELL ACTIVATION (FC)
18
16
14
12
10
8
6
4
2
0
CD38
HLA-DR
CD69


A
1
2
3
ING B
-
24h
48h
CycT1
CDK9
TUBULIN
B
Cyc T1
CDK9
Fold Change (ING treated/non-treated)
0
1
2
3
4
5
24h
48h


A
LUCIFERASE ACTIVITY (FC)
20
15
10
5
0
24h
48h
DBZ (20 nM) + - - + + -
SAHA (330 nM) - + - + - -
SAHA (1000 nM) - - + - + -
B
LUCIFERASE ACTIVITY (FC)
20
15
10
5
0
24h
48h
DBZ (20 nM) + - - + + -
HMBA (330 µM) - + - + - -
HMBA (3000 µM) - - + - + -
C
1 2 3
CycT1
CDK9
Tubulin
D
FOLD CHANGE INTENSITY (TREATED/MOCK)
6
5
4
3
2
1
0
Cyc T1
CDK9
24h
48h