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Supplemental Material to:

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BET bromodomain-targeting compounds reactivate HIV from latency via a Tat-independent mechanism

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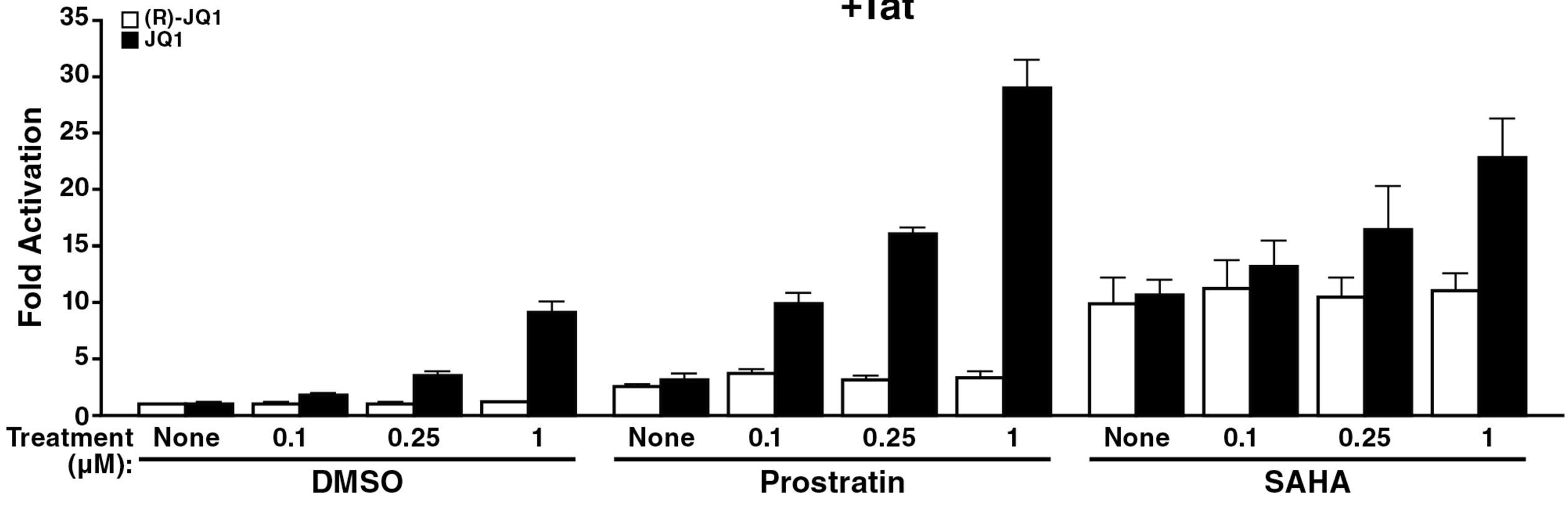
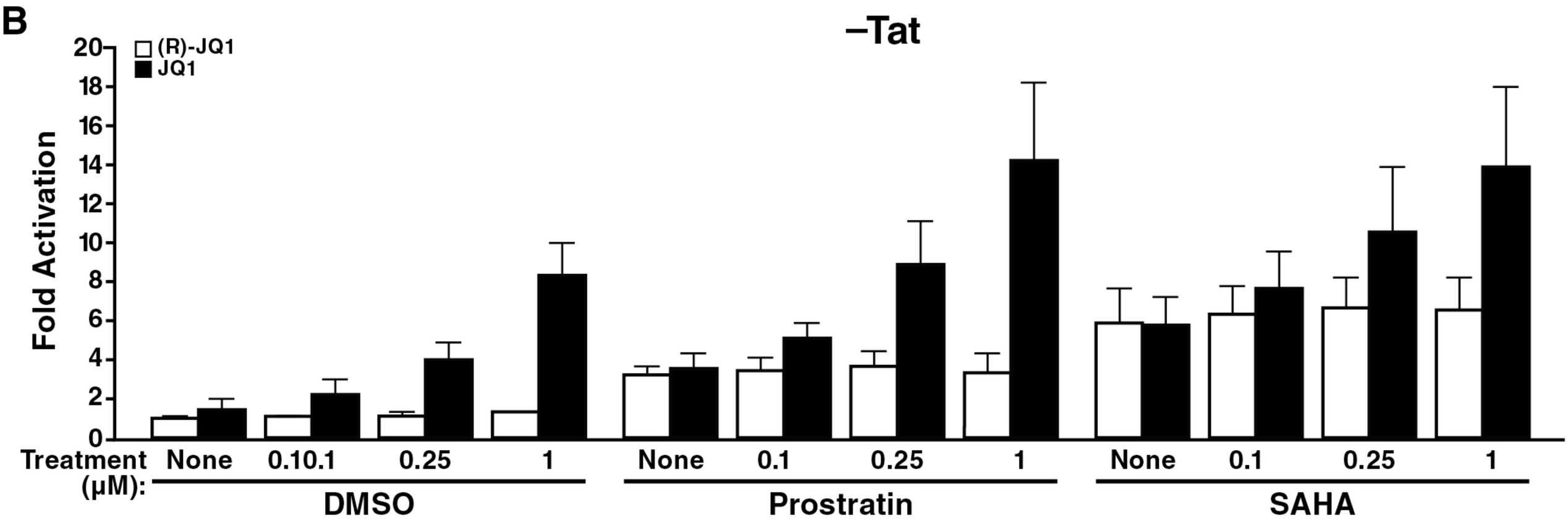
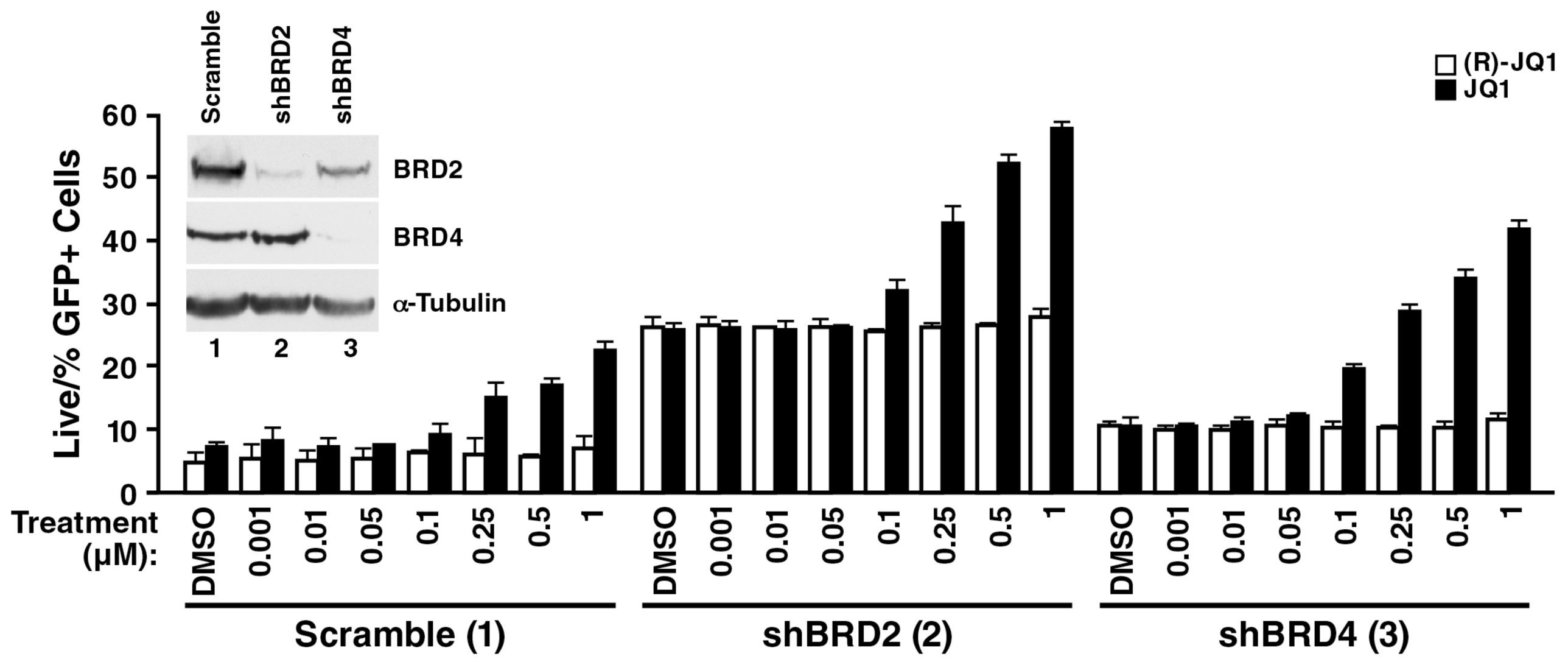
A


+Tat
(R)-JQ1
JQ1
Fold Activation
35
30
25
20
15
10
5
0
Treatment (μM):
None
0.1
0.25
1
None
0.1
0.25
1
None
0.1
0.25
1
DMSO
Prostratin
SAHA

B


–Tat
(R)-JQ1
JQ1
Fold Activation
20
18
16
14
12
10
8
6
4
2
0
Treatment (μM):
None
0.10.1
0.25
1
None
0.1
0.25
1
None
0.1
0.25
1
DMSO
Prostratin
SAHA


Scramble
shBRD2
shBRD4
BRD2
BRD4
α-Tubulin
1
2
3
(R)-JQ1
JQ1
Live/% GFP+ Cells
60
50
40
30
20
10
0
Treatment (μM):
DMSO
0.001
0.01
0.05
0.1
0.25
0.5
1
Scramble (1)
shBRD2 (2)
shBRD4 (3)

Supplementary Material

Supplementary Figure 1

Co-treatment with JQ1 and prostratin activates latent HIV. J-Lat cell lines A2 (**A**) and A72 (**B**) were treated with JQ1 or (R)-JQ1 in combination with prostratin, SAHA, or control at the indicated concentrations for 18 h, followed by flow cytometry analysis. In both cell lines, JQ1 reactivated HIV-1 in a dose-dependent manner, alone or in combination with prostratin or TNFα. The control (R)-JQ1 did not activate the integrated HIV promoter. Results represent average (± SD) of three experiments.

Supplementary Figure 2

Reactivation of latent HIV-1 by inhibition of BRD2 and BRD4. A72 cells were infected with virus containing shRNA constructs targeting different target sequences in BRD2 and BRD4 mRNAs as shown in Figure 7 or a nontargeting control. Four days after infection, cells were treated with JQ1 or DMSO at the indicated concentrations for 18 h, followed by flow cytometry analysis. As indicated, knockdown of BRD2 and BRD4 resulted in an increase in GFP expression. JQ1 treatment enhanced this effect. Results represent average (± SD) of three experiments. Knockdown of BRD2 and BRD4 protein levels were confirmed by immunoblotting with BRD2 and BRD4 antibodies or the control α-Tubulin.