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Paroxetine Intersects with the PKC Pathway and Attenuates the Reactivation of HIV-1 from Latency

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Background

- The major barrier in achieving an HIV-1 cure is the rapid establishment of latent infection[1].
- Functional cure or “Block and Lock”: Target latently infected cells to avoid reactivation of the virus when anti-retroviral therapy (ART) is removed[2].
- In latently-infected cells, the mTORC1 complex, a master regulator of autophagy, a major metabolic process that maintains host cell physiology, severely restricts the reactivation of HIV-1[3].
- Autophagy has been linked to HIV-1 associated neurodegenerative disease (HAND) and the induction of autophagy has been suggested as a functional cure[4,5] .
- Use of anti-depressants, such as Paroxetine and Fluoxetine, has been shown to induce autophagy[6].

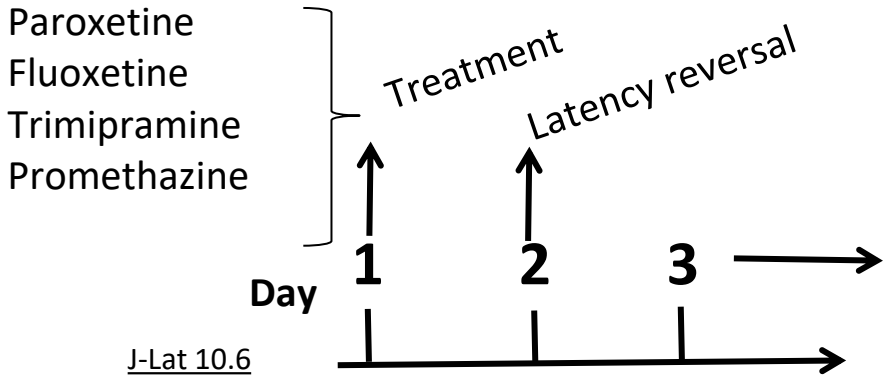
Hypothesis: the induction of autophagy will induce a deep latency and suppress the reactivation of HIV-1 from latently-infected CD4⁺ T cells.

Methodology

Model: J-lat 10.6. Cell line derived from Jurkat that harbour a latent HIV-1 provirus with GFP as a readout, that can be reactivate with several Latency Reversal Agents.

Autophagy-inducing drugs:

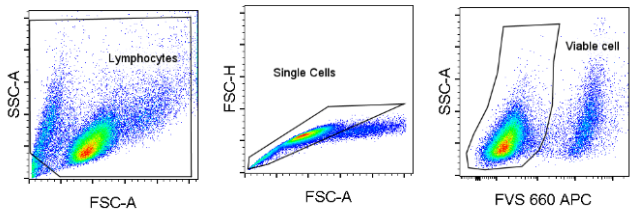
Torin
Paroxetine
Fluoxetine
Trimipramine
Promethazine

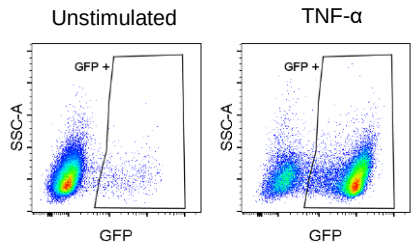


LRA	Activity
PMA Prostratin	PKC activation
TNF-α	TRAF recruitment
SAHA	HDAC inhibition

Readout:

- % of reactivated cells (GFP⁺) – Flow cytometry





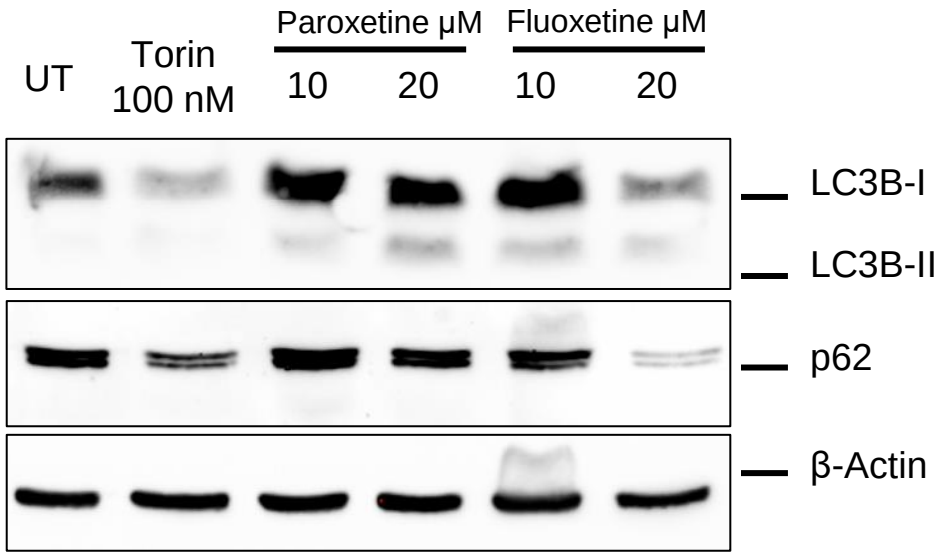


Fig 1. Paroxetine and Fluoxetine induce autophagy flux

J-lat were treated with the drugs and the lysate were collected after 24 hours and the membrane was stained for LC3B, p62 and β-Actin. Conversion of LC3B I to II indicates autophagy induction and reduction of p62 reflects the degradation, thus, autophagy flux.

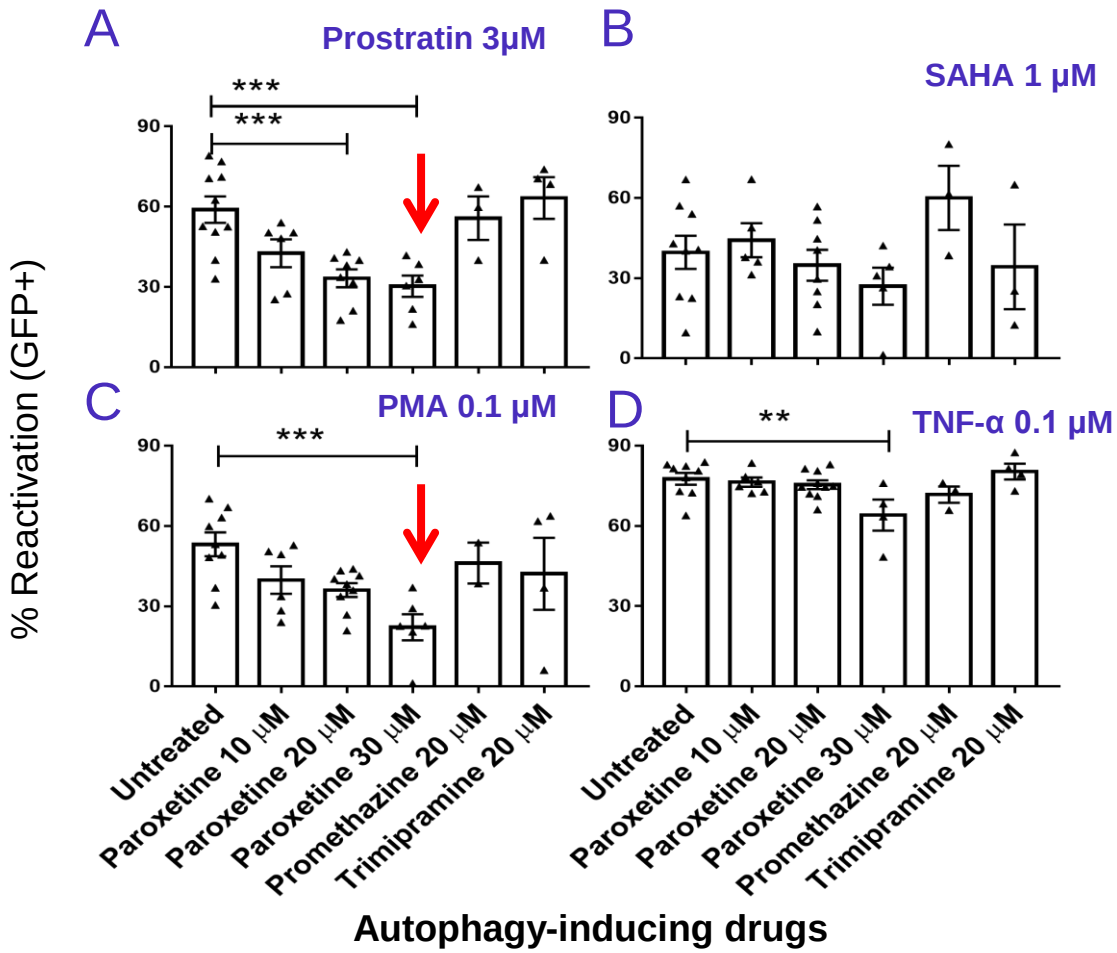
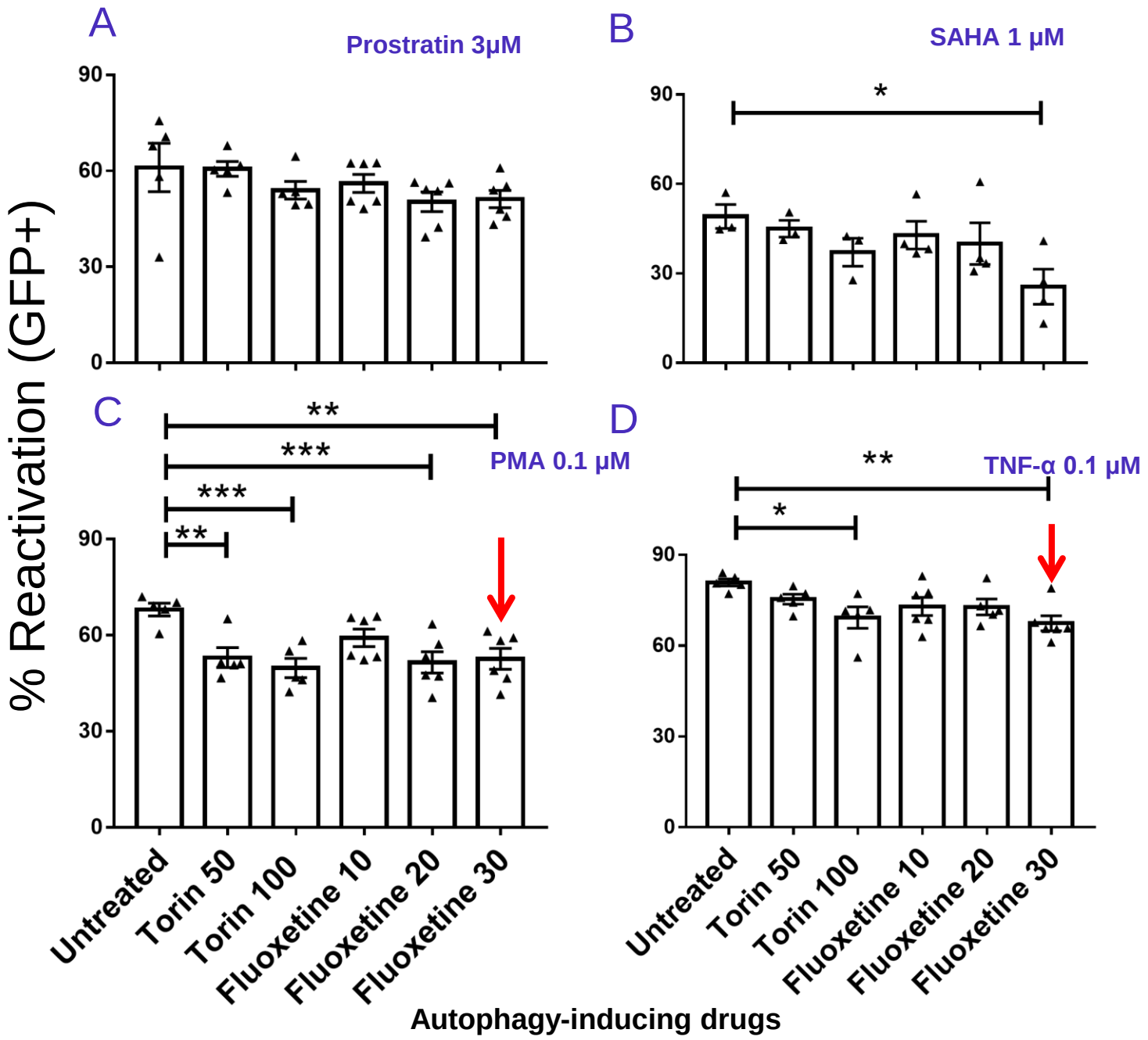


Fig 2 . Paroxetine attenuates the reactivation of provirus

J-lat were treated with the autophagy inducing drugs for 24 hours and then reactivated with (A) Prostratin 3 μM, (B) SAHA 1 μM, (C) PMA 0.1 μM, (D) TNF-α 0.1 μM for 24 hours and analysed for GFP expression through Flow Cytometry. Data show percentage (%) of positive cells for GFP and presented as Mean ± SEM. Statistical analysis was performed with ANOVA one way with post Dunnett's test. **, *** as $p < 0.01$ and 0.001

Fig 3 . Fluoxetine attenuates the reactivation of provirus

J-lat were treated with the autophagy inducing drugs (Torin and Fluoxetine) for 24 hours and then reactivated with (A) Prostratin 3 µM, (B) SAHA 1 µM, (C) PMA 0.1 µM, (D) TNF-α 0.1 µM for 24 hours and analysed for GFP expression through Flow Cytometry. Data show percentage (%) of positive cells for GFP and presented as Mean ± SEM. Statistical analysis was performed with ANOVA one way with post Dunnett's test. **, *** as $p < 0.01$ and 0.001



Conclusion

- Paroxetine and Fluoxetine induce autophagy in latently infected T cells.
- Paroxetine and Fluoxetine reduce the reactivation of HIV-1 in latently infected T cells.
- Paroxetine intersects with PKC pathway to reduce the reactivation of the provirus

Relevance

- This work has the potential to inform on therapeutics for HAND and functional cure strategies by repurposing FDA-approved drugs for HIV-1.

Reference

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Acknowledgemets

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