# Supplementary Materials for

## The HIV latency reversing agent HODHBt inhibits the phosphatases PTPN1 and PTPN2

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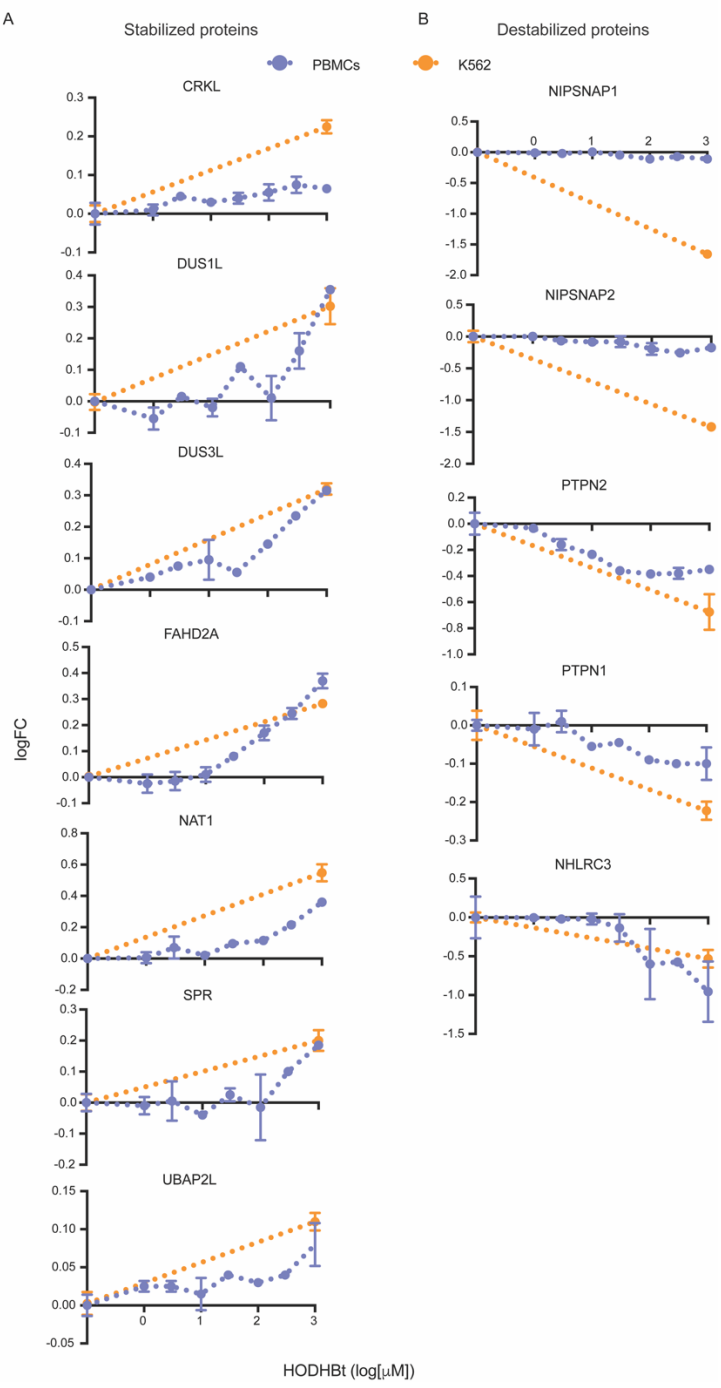
**The PDF file includes:**

Supplemental Figures 1-7

**Other Supplementary Materials for this manuscript include the following:**

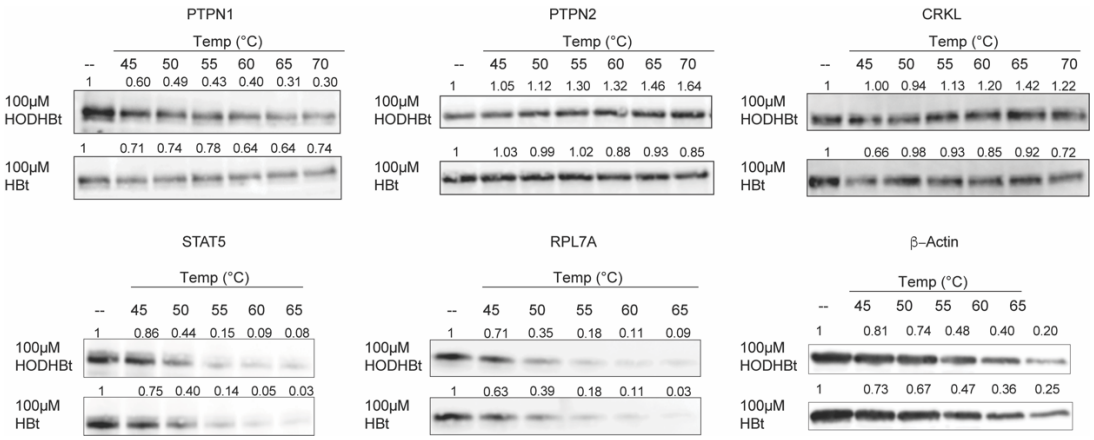
Supplemental Data Files 1-3

## Supplemental Figures

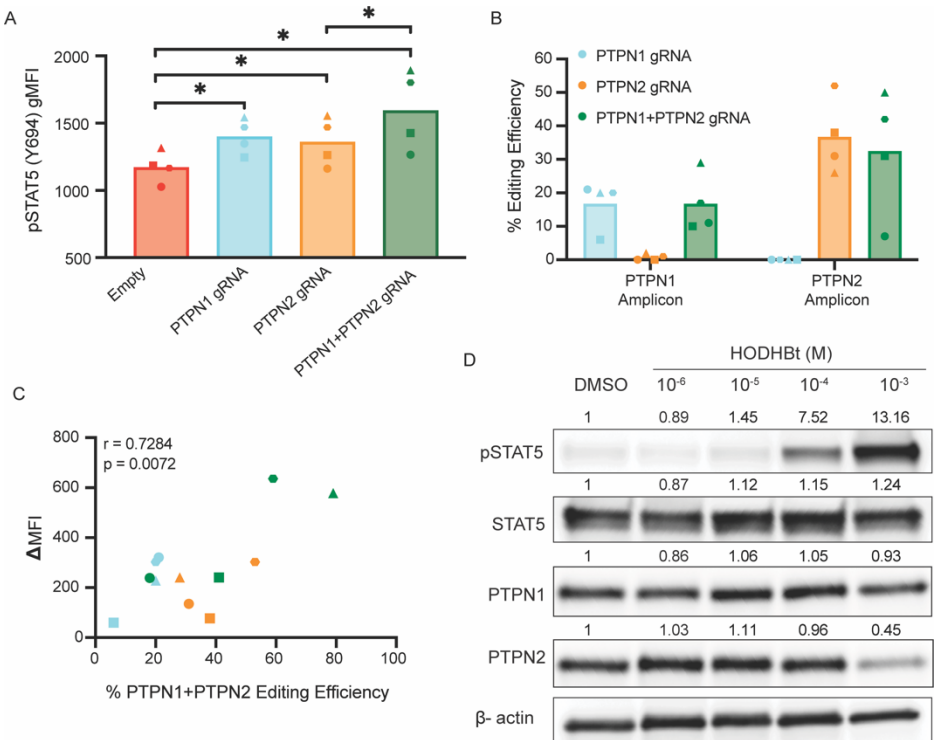



**Supplemental Figure 1. Concentration-response curves for common proteins with changes in thermal stability for PBMCs and K562 cells in the TPP.**

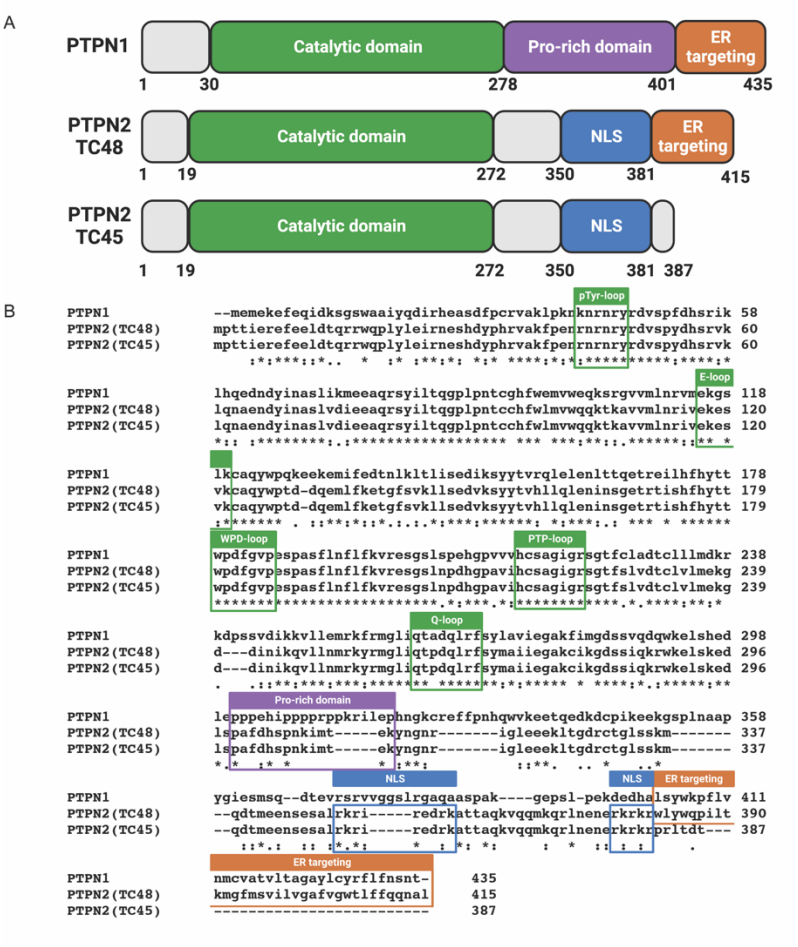
X-axis is $\log_{10}$-transformed compound concentration and y-axis is the log2FC of the peptide presence relative to the vehicle concentration calculated as indicated in the Materials and Methods. (**A**) Stabilized proteins. (**B**) Destabilized proteins.




**Supplemental Figure 2. Representative CETSA western blots.**
Analysis of CETSA thermal shifts for proteins of interest in CD4 T cell lysates. Data representative of 2-4 western blots per protein.




**Supplemental Figure 3. PTPN1 and PTPN2 deletion increases STAT5 phosphorylation.** (**A**) pSTAT5 levels in K562 cells after CRISPR/Cas9 knockout of PTPN1, PTPN2, or PTPN1/PTPN2 (n=4). Paired t-test used to calculate p-values (*p<0.05). (**B**) Editing efficiencies from the 4 independent experiments for each amplicon measured using T7 Endonuclease digestion. (**C**) Correlations between pSTAT5 ΔMFI (guide RNA minus Cas9 alone) for each experiment and combined knockout editing efficiency. Pearson correlation coefficients calculated. (**D**) Analysis of STAT phosphorylation and levels of PTPN1 and PTPN2 in K562 cells after HODHBt dose response treatment for 24 hours.




**Supplemental Figure 4. Sequence similarity of PTPN1 and PTPN2.**
(**A**) Schematic representation of PTPN1 and PTPN2. (**B**) Sequence alignment between PTPN1 and the two PTPN2 isoforms. The catalytic domains retain 98% similarity between proteins. Nuclear localization sequence (NLS) allows for nuclear translocation of PTPN2. Created with Biorender.com

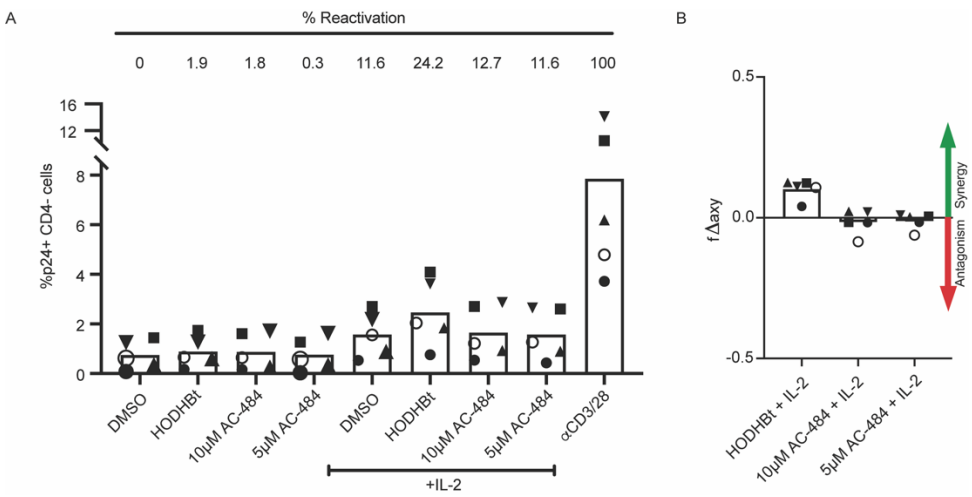
PTPN1 (1-321)

| [Inhibitor] | Vmax | Km |
|---|---|---|
| DMSO | 233.5 | 4.52 |
| 250μM HODHBt | 189.3 | 7.34 |
| 500μM HODHBt | 151.5 | 7.78 |
| 1mM HODHBt | 135 | 8.31 |

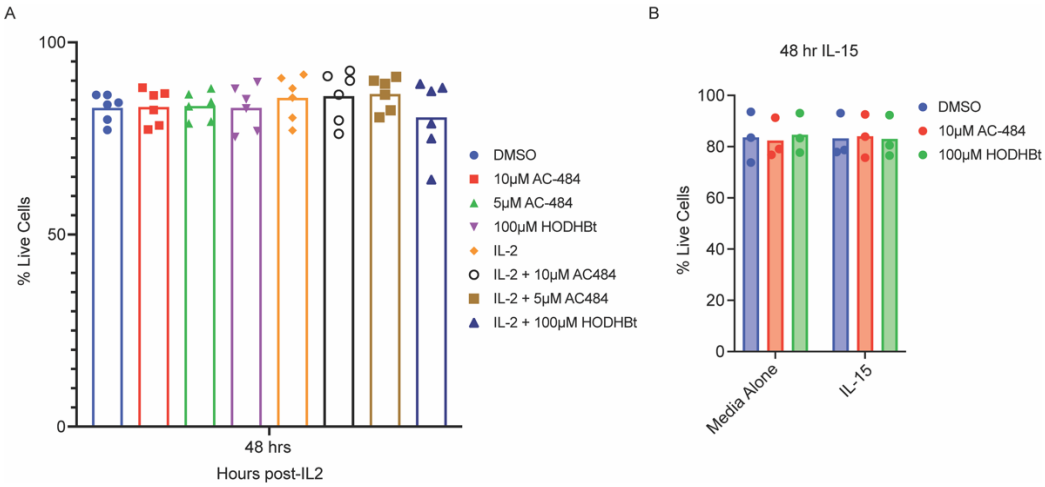
PTPN2 (2-315)

| [Inhibitor] | Vmax | Km |
|---|---|---|
| DMSO | 1.08 | 11.8 |
| 250μM HODHBt | 0.76 | 12.89 |
| 500μM HODHBt | 0.71 | 12.49 |
| 1mM HODHBt | 0.42 | 9.25 |

**Supplemental Figure 5. Enzymatic kinetic values for HODHBt against PTPNs.**
Enzyme kinetic values for HODHBt against PTPN1 catalytic domain and PTPN2 catalytic domain. Calculated using Michaelis-Menten equation analysis in Prism 9 for Mac OS X software (GraphPad Software, Inc., La Jolla, CA)




**Supplemental Figure 6. AC-484 has limited reactivation activity with IL-2.**
(**A**) Reactivation of latent HIV in $T_{CM}$ measured by flow cytometry after treatment with 100μM HODHBt or 10μM AC-484 +/- 30 IU/mL IL-2, or $\alpha$CD3/CD28 (n=5). (**B**) Bliss independence synergy calculations for reactivation.




**Supplemental Figure 7. Treatment with HODHBt and AC-484 has no effect on viability of primary CD4 T cells.**
Viability of total CD4 T cells after pre-treatment for 2 hours with HODHBt and AC-484, and 48 hours in culture with and without IL-2 (n=6) (**A**) and IL-15 (n=3) (**B**).

**Data S1. Raw data from Pelago CETSA-MS analysis of HODHBt**
Intact PBMC and K562 data sets after treatment with HODHBt and subsequent analysis by LC-MS.

**Data S2. Common proteins between PBMC and K562 data sets**
Filtered for significance ($p>0.01$). Stabilized and destabilized proteins for each data set and common proteins between the two.

**Data S3. STRING interaction analysis of common proteins**
Interactions between common proteins from CETSA-MS analysis and STAT5.