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Supplementary Materials for

Targeting CCR5 trafficking to inhibit HIV-1 infection

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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Supplementary figure legends

Figure S1

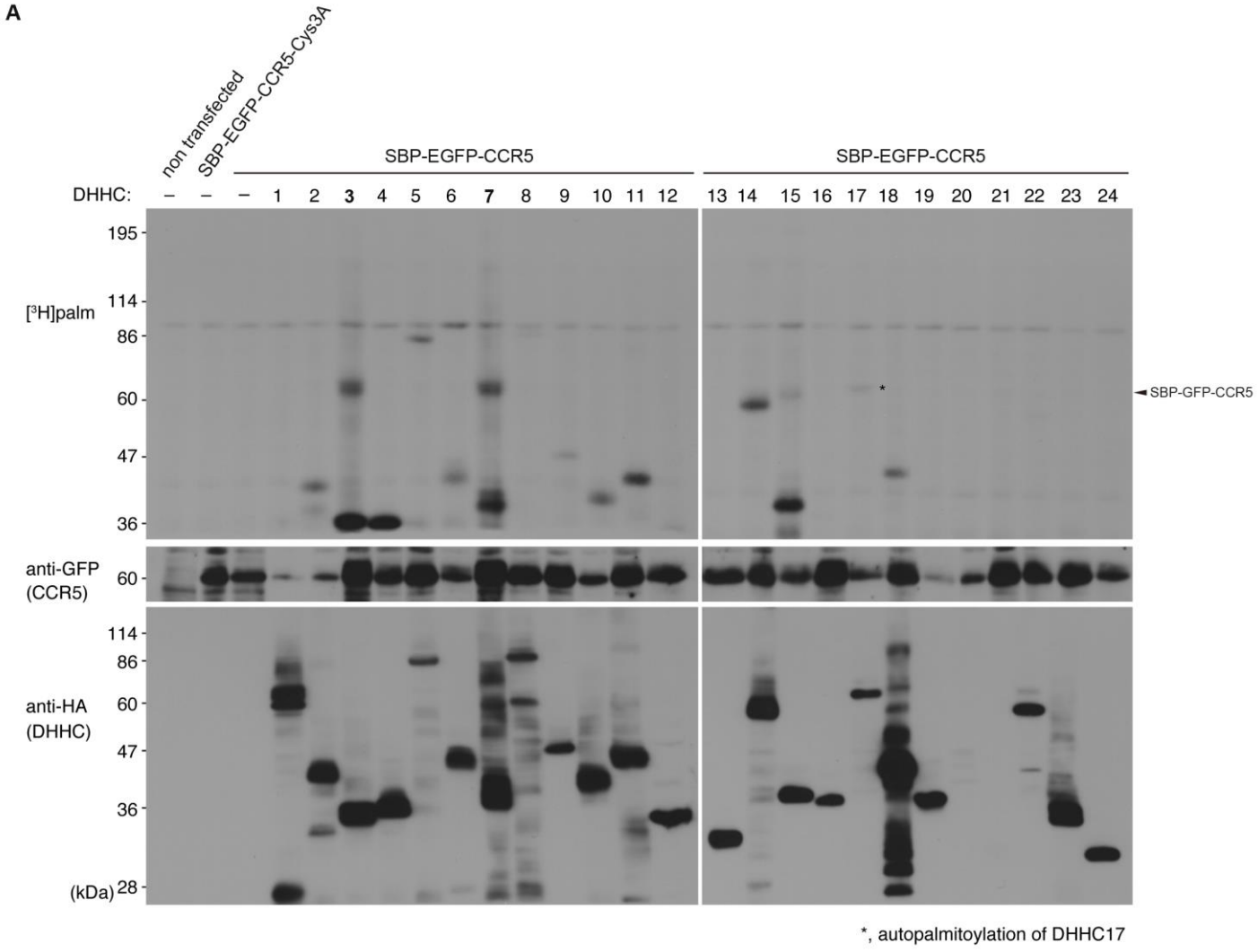

Fig. S1. Molecules 13 and 14 inhibit autopalmitoylation of DHHC3 and DHHC7. (A) HEK293T cells were transfected with SBP-EGFP-CCR5 Cys3A or with the indicated HA-DHHCs and SBP-EGFP-CCR5. 24 h after transfection, cells were labeled with [3H]palmitate (0.5 mCi/ml) for 4 hr. **(B)** HEK293T cells were transfected with the HA-DHHC3, HA-DHHC7 or their respective catalytically inactive mutants HA-DHCS3 and HA-DHCS7. 24 h after transfection, cells were labeled with [3H]palmitate (0.5 mCi/ml) for 4 hr. Cells were incubated with 10 uM of individual compounds for 30 min before the labeling and for 4h together with [3H]palmitate. For compund (-), DMSO was added. For the fluorography, after SDS-PAGE of cell lysates, the gels were exposed to the film for 14-43 h. **(C)** N = 3 independent experiments. The mean ± sem is shown. *$p < 0.05$, non-parametric Kruskal-Wallis test and Steel multiple comparison

Figure S2

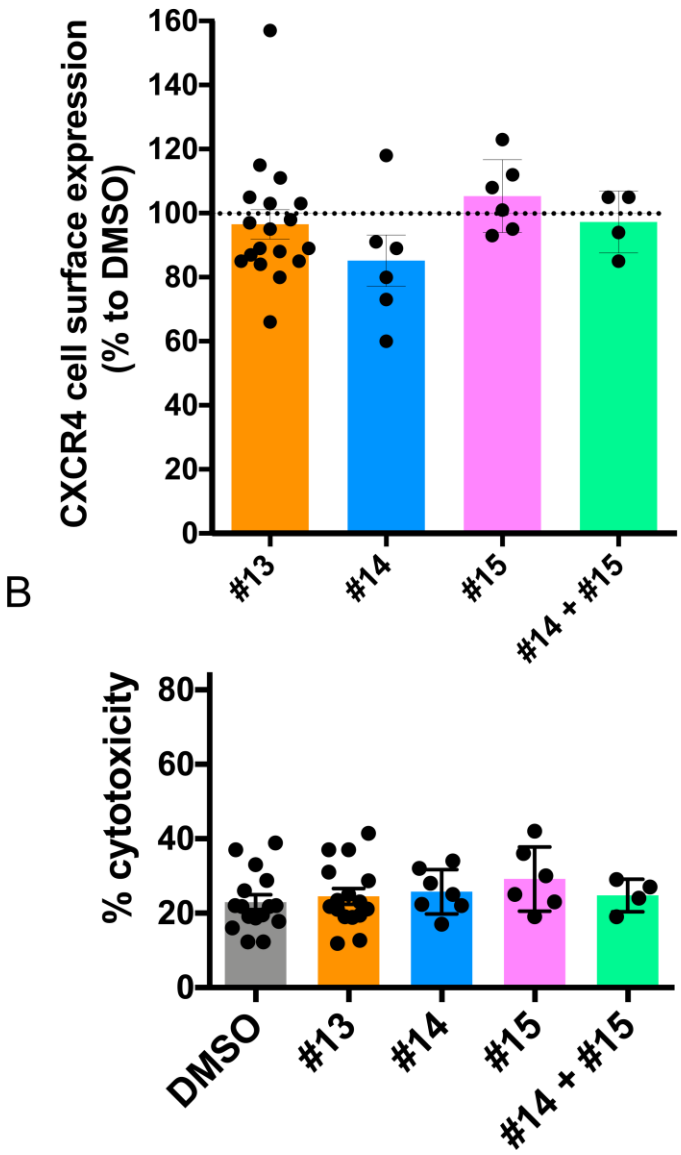

Fig. S2. Molecules 13, 14, and 15 do not alter CXCR4 surface expression or induce cytotoxicity in primary macrophages. Primary human macrophages differentiated for 4 days with rhM-CSF were treated during 18 h with molecule 13 at 10 µM; molecule 14 at 3 µM; molecule 15 at 1 µM and molecules 14 and 15 at 1 µM (or DMSO at 0.1%). **(A)** Cell surface expression of CXCR4 was measured by flow cytometry with specific antibodies. **(B)** Cytotoxicity was evaluated by measuring LDH release in human primary macrophages after 18 h pre-treatment with molecules or DMSO as a control.

Movie S1. Synchronized transport of CCR5. HeLa cells stably expressing Str-KDEL_SBP-EGFP-CCR5 imaged with a spinning disk microscope. Time is indicated in min:sec. Trafficking was induced by addition of biotin at 00:00. The image displayed corresponds to z-projection. Movie S1 corresponds to Fig.1a.

Movie S2. Synchronized transport of TNF. HeLa cells stably expressing Str-KDEL_TNF-SBP-EGFP imaged with a spinning disk microscope. Time is indicated in min:sec. Trafficking was induced by addition of biotin at 00:00. The image displayed corresponds to z-projection. Movie S2 corresponds to Fig 1a.

Movie S3. Synchronized transport of CCR5 and TNF. HeLa cells co-expressing Str-KDEL_SBP-EGFP-CCR5 and Str-KDEL_TNF-SBP-mCherry were imaged with a spinning disk microscope. Time is indicated in min:sec. Trafficking was induced by addition of biotin at 00:00. The image displayed corresponds to z-projection. Movie S3 corresponds to Fig 1c.