



Supplementary Fig. 1. Analysis of freshly isolated human primary  $\beta$ 7+CD4+ T cells by flow cytometry. (a) Gating strategy for measuring the expression of  $\alpha$ 4 and  $\alpha$ E on human primary  $\beta$ 7+CD4+ T cells. (b) Expression of Ki67, CD25 and CD69 on human primary  $\beta$ 7+CD4+ T cells (n=5). Data are expressed as means ± SEM.



Supplementary Fig. 2. Gating strategy for measuring primary or RA-induced  $\alpha4\beta7^{+}$   $_{\text{MEM}}T$  cells by flow cytometry.



Supplementary Fig. 3. Measuring the percentages of MAdCAM-1-Fc bound Primary  $\alpha 4\beta 7^{+}$  memory CD4<sup>+</sup> T cells and  $\alpha 4\beta 7^{+}_{MEM}$ T by flow cytometry. Data are expressed as means  $\pm$  SEM (n=5).



Supplementary Fig. 4. VDZ does not further upregulate the expression of Ki67, CD25 and CD69 on RA-induced  $\alpha 4\beta7^+$ MEMT cells. The expression of Ki67, CD25 and CD69 on RA-induced  $\alpha 4\beta7^+$  MEMT cells was measured by flow cytometry after being cultured in the presence or absence of VDZ at 37°C for 24 hours. Data are presented as means  $\pm$  SEM (n=5).



Supplementary Fig. 5. Anti-hulgG1 does not further upregulate the expression of CD25 and CD69 on VDZ-treated  $\alpha 4\beta 7^+_{MEM}T$  cells. The expression of CD25 and CD69 on VDZ-treated  $\alpha 4\beta 7^+_{MEM}T$  was measured by flow cytometry after being cultured in the presence or absence of anti-hulgG1 at  $37^{\circ}C$  for 24 hours. Data are expressed as means ± SEM (n=5).



Supplementary Fig. 6. Binding of pseudotyped HIV-1<sub>SF162</sub> to  $\alpha 4\beta7$ -expressing cells *in vitro*. (a) Binding of pseudotyped HIV-1<sub>SF162</sub>-GFP to RA-induced  $\alpha 4\beta7^+_{MEM}T$  cells was determined in the presence of 1 mM Mn<sup>2+</sup> at 4<sup>o</sup>C for 1 hour before being subjected to flow cytometry analysis. The Mu-Act-1 antibody only partially blocked the viral binding activity. (b) Expression of  $\alpha 4\beta7$  heterodimers on 293T cells co-transfected transiently with  $\alpha$ 4- and  $\beta7$ -expressing plasmids. (c) Binding of pseudotyped HIV-1<sub>SF162</sub>-GFP to  $\alpha 4\beta7$ -expressing 293T cells with or without 1 mM Mn<sup>2+</sup> and the Mu-Act-1 antibody. Consistently, the Mu-Act-1 antibody partially blocked the viral binding activity.



Supplementary Fig. 7. Representative images of VDZ-induced homotypic aggregation of RA-induced  $\alpha 4\beta7^+$  <sub>MEM</sub>T cells before and post HIV-1<sub>SF162</sub> entry. Images were taken one hour after VDZ was added into the cell cultures.



Supplementary Fig. 8. Populations of human immune cells in NSG mice after cell transfusion. (a) Frequencies of human T cells in the peripheral blood of NSG-HuPBL mice (n=14) were detected by flow cytometry three weeks post human PBMC transfusion. (b) Frequencies of human immune cell subsets in the peripheral blood of NSG-CD34 mice (n=23) were detected by flow cytometry 15 weeks after CD34<sup>+</sup> cell transfusion. (c) Frequencies of peripheral  $\beta$ 7<sup>+</sup>CD4<sup>+</sup> T cells in NSG-HuPBL mice (n=14) and NSG-HuCD34 mice (n=15) were determined at the day before HIV-1 challenge. (d) Frequencies of peripheral CCR5<sup>+</sup>CD4<sup>+</sup> T cells in NSG-HuPBL mice (n=11) and NSG-HuCD34 mice (n=25) were determined at the day before HIV-1 challenge. Data are expressed as means ± SEM.



Supplementary Fig. 9. Distribution of  $\alpha 4\beta 7^+CD4^+ T$  cells in the lamina propria of humanized mice. (a) Representative immunohistochemical staining of CD3<sup>+</sup> T cells (red) in the small intestine of NSG-HuPBL and NSG-HuCD34 mice. (b) The frequency of  $\alpha 4\beta 7^+CD4^+$  T cells in the lamina propria of small intestine of humanized mice (n=5) was measured by flow cytometry. (c) Expression of  $\alpha 4$  and  $\alpha E$  on human  $\beta 7^+CD4^+$  T cells on the lamina propria of humanized mice were analyzed. Data are expressed as means  $\pm$  SEM.



Supplementary Fig. 10. Blocking activity and half-life of VDZ in NSG-HuPBL mice. (a) Representative flow cytometric plots showing the blockade of VDZ-647 binding towards  $\alpha 4\beta7$  heterodimers on freshly isolated  $\alpha 4\beta7^+_{MEM}T$  cells at day 0, 1 and 4 after VDZ injection. (b) Representative flow cytometric plots showing the blockade of MAdCAM-1 binding towards  $\alpha 4\beta7$  heterodimers on freshly isolated  $\alpha 4\beta7^+_{MEM}T$  cells at day 0, 1 and 4 after VDZ injection. (c) and (d) present percentages and statistical analysis of the blockade effects described in (a) and (b), respectively. (e) The in vivo half-life of VDZ in the plasma of the humanized mice, as determined by ELISA. Data are shown as means  $\pm$  SEM (*n*=5).



Supplementary Fig. 11. VDZ does not prevent HIV-1 infection in humanized NSG-HuPBL mice. (a) Experimental design and schedule. (b) Plasma viral RNA copies were determined by qPCR over time as means  $\pm$  SEM (left) and in individual animals (right). (c) Frequencies of human CD4<sup>+</sup> T cells were measured by flow cytometry over time as means  $\pm$  SEM (left) and in individual animals (right). (d) Frequencies of human  $\beta7^+$  CD4<sup>+</sup> T cells were determined by flow cytometry over time as means  $\pm$  SEM (left) and in individual animals (right). (e) The frequency of P24<sup>+</sup> cells in total CD4<sup>+</sup> T cells and  $\alpha4\beta7^+$ CD4<sup>+</sup> T cells in NSG-huPBL mice were determined by flow cytometry three weeks post HIV-1<sub>SF162</sub> infection. Data are shown as means  $\pm$  SEM (n=5)



Supplementary Fig. 12. Blockade of  $\alpha 4\beta 7$  by VDZ on peripheral  $\beta 7^+CD4^+$  T cells in humanized NSG-HuCD34 mice 20 days after the final dose of VDZ injection.



Supplementary Fig. 13. Measuring anti-VDZ IgM (a)/anti-RM-Act-1 IgM (b) in the plasma of NSG-HuCD34 mice after treated with or without VDZ/RM-Act-1 for a total of seven times by ELISA. Plasma from non-humanized NSG mice was used as a control. Data are expressed as Mean  $\pm$  SEM (n=4).



**Supplementary Fig. 14.** The blockade efficacies of VDZ/RM-Act-1 on  $\alpha 4\beta 7^+$ T cells were assessed 10 days post the final infusion of VDZ/RM-Act-1. Error bars indicate means ± SEM.