

Supplementary Figure 3:  $\alpha 4$  and  $\beta 7$  integrin expression on  $T_{Reg}$  and  $T_{Eff}$  cells, vedolizumab binding to memory CD4<sup>+</sup> T cells and controls for VDZ binding.

- (A) Quantitative flow cytometry of  $\alpha 4^+\beta 7^+$  T<sub>Reg</sub> and T<sub>Eff</sub> cells in patients with UC and CD compared with healthy controls. n = 17-28 IBD patients or healthy controls as indicated.
- (B) Mean fluorescence intensity (MFI) of  $\alpha 4$  and  $\beta 7$  integrin on  $T_{Reg}$  and  $T_{Eff}$  cells in patients with UC and CD an in healthy controls. n = 17-28 IBD patients or healthy controls as indicated.
- (C) Quantitative flow cytometry of VDZ+ cells after gating on  $\alpha 4\beta 7^+$  CD45RO+  $T_{Reg}$  and  $T_{Eff}$  cells following incubation with the indicated concentrations of fluorescently labelled vedolizumab. Quantitative data are expressed relative to  $T_{Eff}$  cells. n=25 IBD patients and healthy controls.
- (D) Representative fluorescence microscopy of FACS-purified  $T_{Reg}$  and  $T_{Eff}$  cells stained with isotype controls for  $\beta 7$  antibody (green) and VDZ (red) and counterstained with Hoechst (blue); Scale bar 10  $\mu$ m. Representative images from 1 out of 6 independent experiments (cells purified from leukocyte cones).

Statistical comparisons were performed using paired t-test (A, C), 2way ANOVA with Sidak's multiple comparison test (B)

Sample donor characteristics are listed in *Supplementary Table S13*.