Supplementary Information

Th1-like state of CD4$^+$CCR4$^+$ T-cells in HTLV-1-associated myelopathy

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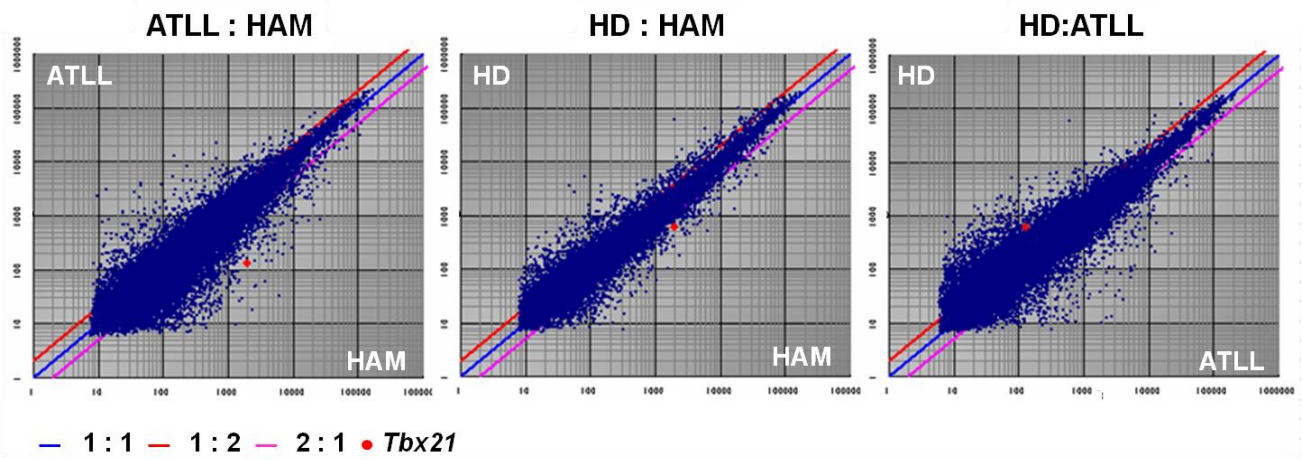
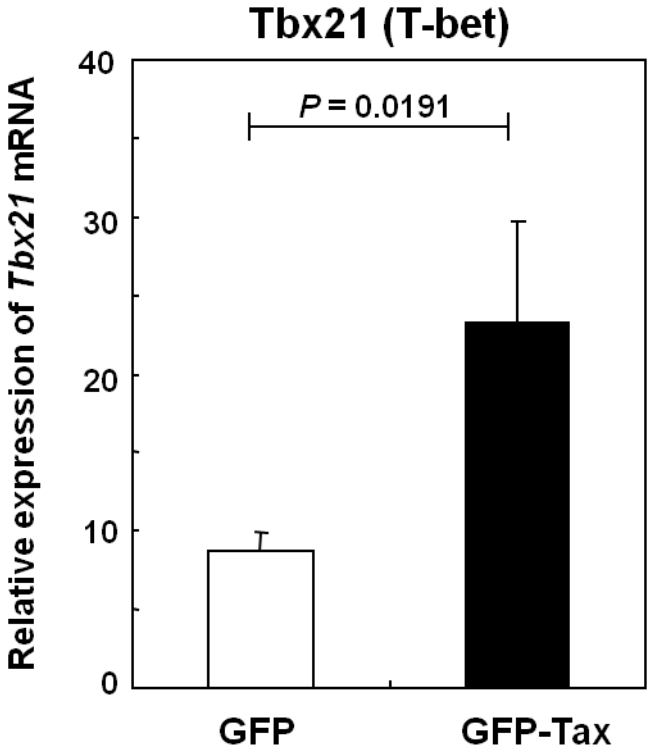


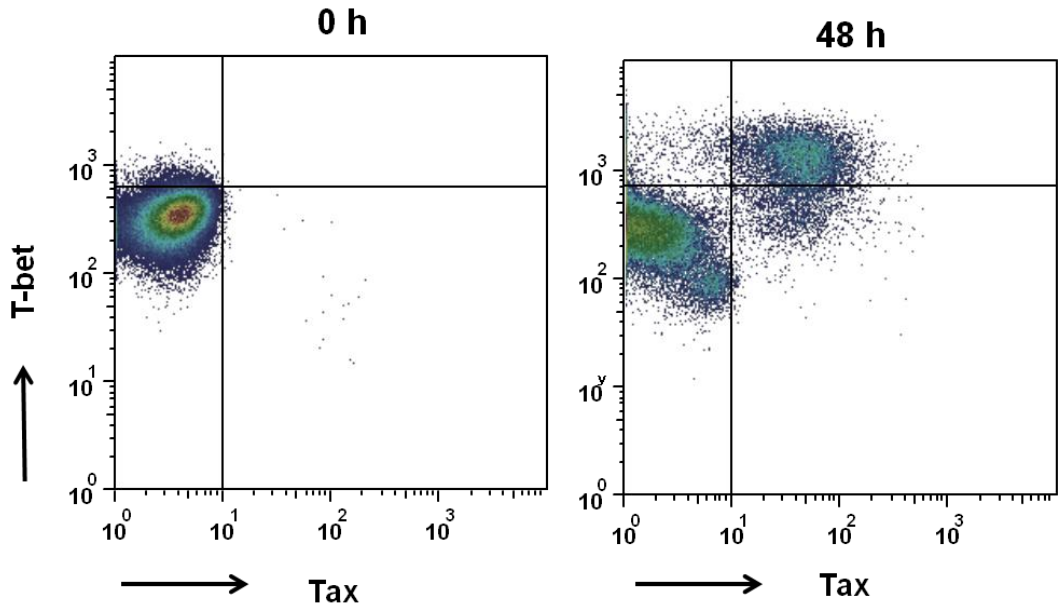
Supplemental Figure 1

***Tbx21* expression in CD4$^+$CD25$^+$CCR4$^+$ T-cells is up-regulated in HAM/TSP patients.** Scatter plots comparing the ratios of signal values from microarray hybridizations with Cy3-labeled mRNAs. The gene expression profiles of CD4$^+$CD25$^+$CCR4$^+$ T-cells from an HD, an ATLL patient, and a HAM/TSP patient were compared in order to identify target molecules for HTLV-1 Tax, with particular emphasis on genes known to be associated with IFN-γ production. DNA microarray analysis revealed that *Tbx21* was heavily up-regulated in the HAM/TSP patient with respect to both the HD and the ATL patient. Data was analyzed using GeneSpringGX. From left to right, the panels compare the ATLL vs. HAM/TSP, HD vs. HAM/TSP, and HD vs. ATLL. The colored dots and lines signify the following: each dot represents a gene, with the red dot highlighting the *Tbx21* gene; the 2:1, 1:1, and 1:2 ratio lines are shown in red, blue, and pink, respectively.



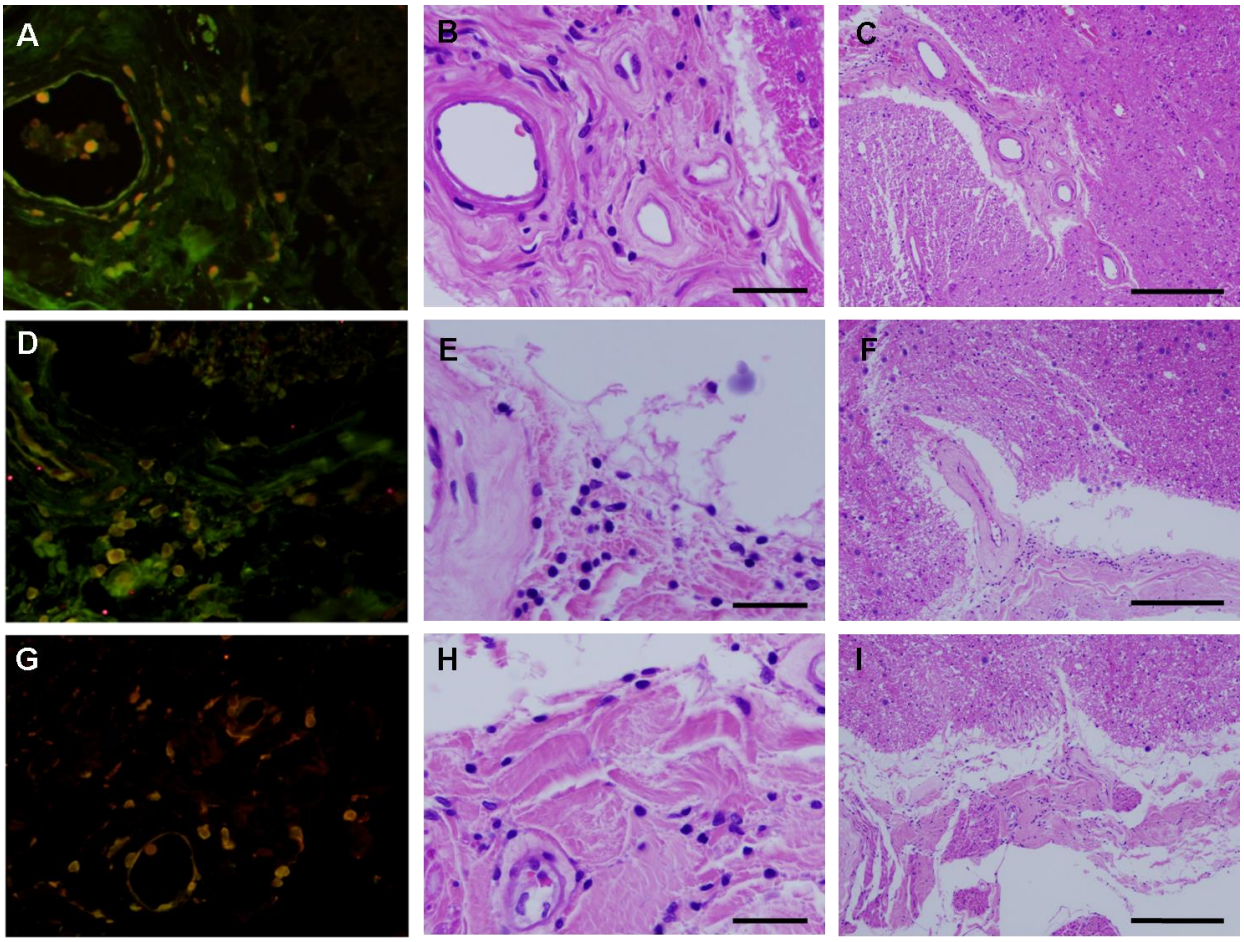
Supplemental Figure 2

Tax expression up-regulates *Tbx21* expression in uninfected $CD4^+CD25^+CCR4^+$ T-cells. PBMCs from an HD were isolated and sorted via FACS, then the $CD4^+CD25^+CCR4^+$ T-cells were incubated with the T_{reg} Suppression Inspector for 38 h and then infected with a lentivirus expressing GFP or GFP-Tax. After 24 h, total RNA was prepared from the cultured cells, and *Tbx21* mRNA expression level was analyzed using real time RT-PCR. Error bars represent the mean ± SD. Statistical analyses were performed using the unpaired *t*-test.



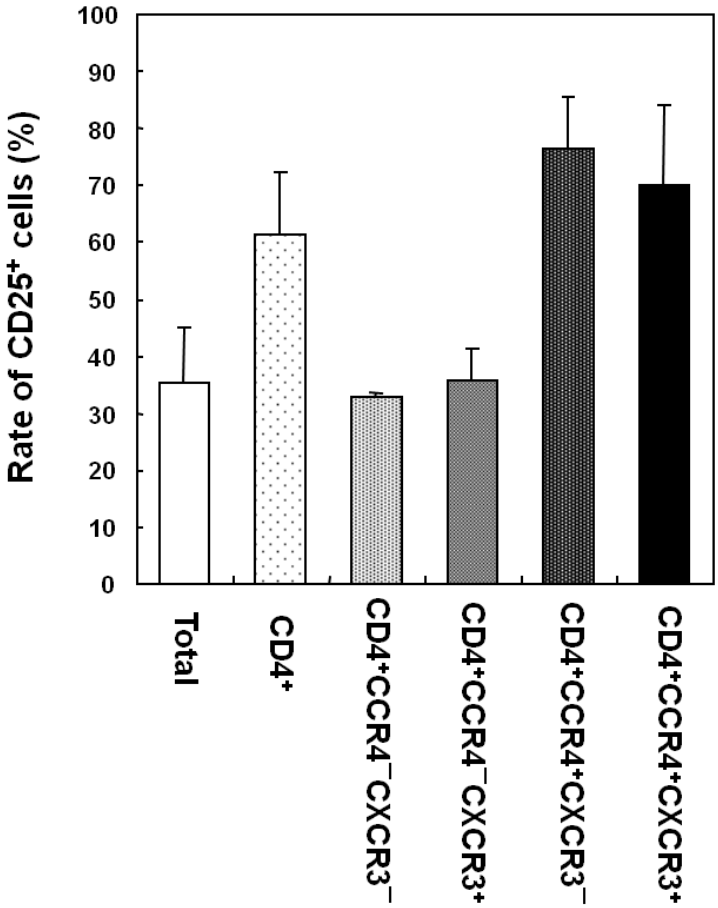
Supplemental Figure 3

Infected CD4$^+$CCR4$^+$ cells in culture begin expressing Tax accompanied by T-bet. Shown above is a representative dot plot of Tax versus T-bet expression in CD4$^+$CCR4$^+$ cells isolated from a HAM/TSP patient and then labeled with fluorescent-conjugated antibodies for Tax and T-bet immediately and after 48 h of culturing.



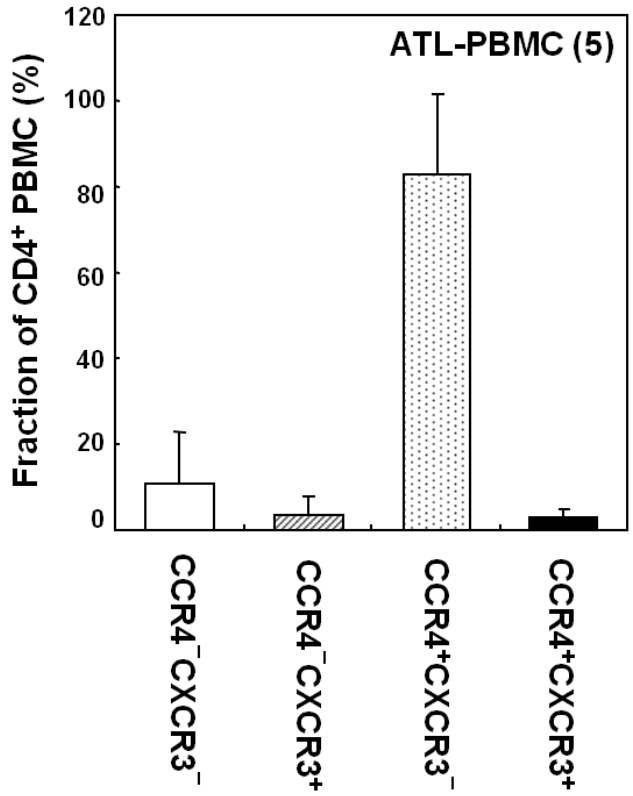
Supplemental Figure 4

Lymphocytic Infiltrates in the Spinal Cord of a HAM/TSP Patient. Shown above are images of Haematoxylin and eosin (H&E)-stained spinal cord tissue sections corresponding to the immunofluorescent images shown in Fig 4A: co-detection of CCR4 and T-bet (A-C), CCR4 and IFN-γ (D-F), and CCR4 and CXCR3 (G-I). The images from Fig. 4A are included for comparison (A, D, G), followed by H&E staining at the same magnification (B, E, H, Scale bars: 50 μm), and finally H&E staining at a lower magnification to observe the surrounding area (C, F, I, Scale bars: 200 μm). The H&E staining shows perivascular infiltrates of lymphocytes.



Supplemental Figure 5

The presence of $CD4^+CCR4^+CXCR3^+CD25^+$ cells in the CSF of HAM/TSP patients is substantial. The graph shows the rate of CD25 positivity in each $CD4^+$ cell population in the CSF of HAM/TSP patients (n = 3). The majority (70%) of $CD4^+CCR4^+CXCR3^+$ cells were $CD25^+$. These percentages were calculated using FACS. Error bars represent the mean ± SD.



Supplemental Figure 6

CD4$^+$ PBMCs in ATLL patients are mostly CCR4$^+$CXCR3$^–$, with almost no CCR4$^+$CXCR3$^+$ cells. Graph shows the percentages of CCR4$^–$CXCR3$^–$, CCR4$^–$CXCR3$^+$, CCR4$^+$CXCR3$^–$ and CCR4$^+$CXCR3$^+$ T-cells among PBMCs isolated from ATLL patients (n = 5). Analysis was performed using FACS. Error bars represent the mean ± SD.