

Supplementary Materials: Heat Shock Enhances the Expression of the Human T Cell Leukemia Virus Type-I (HTLV-I) Trans-Activator (Tax) Antigen in Human HTLV-I-Infected Primary and Cultured T Cells

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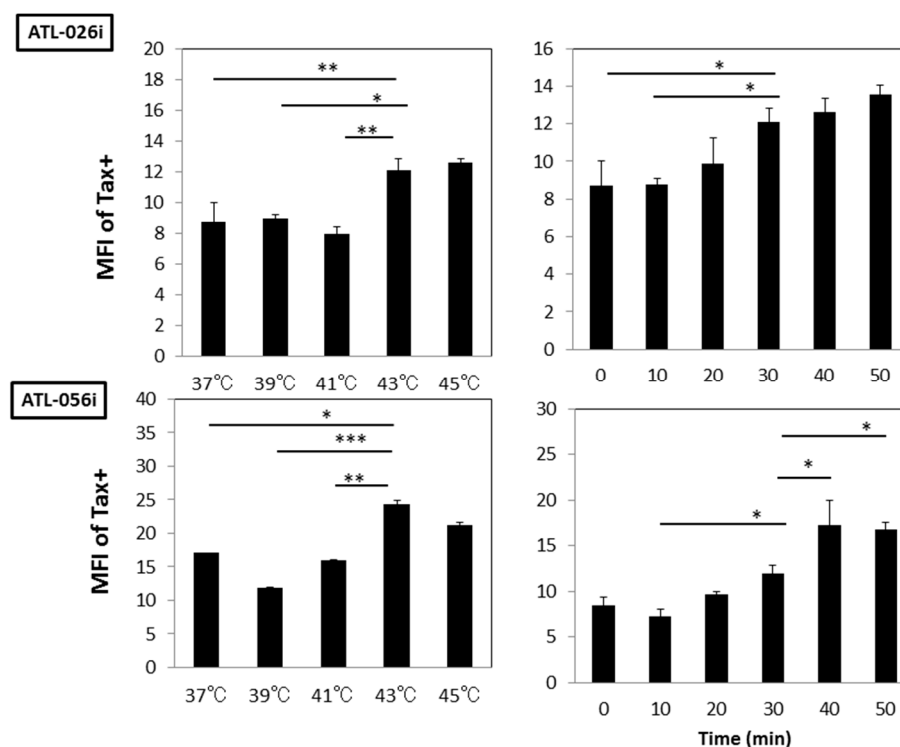


Figure S1. HS increased the MFI of Tax⁺ cells: HTLV-I-infected cell lines, ATL-026i and ATL-056i, were either mock treated (37 °C) or exposed to heat stress at 39–45 °C or for 30 min or at 43 °C for 10–50 min. Tax expression at 24 h after heat shock (HS) is shown. The values denote the means ± SD. **p*<0.05, ***p*<0.01, ****p*<0.001.

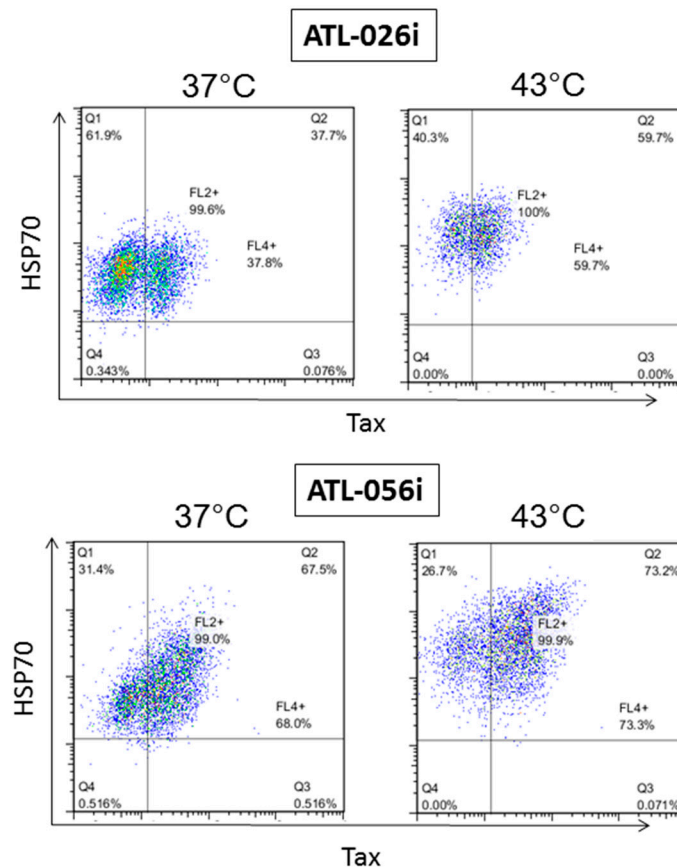


Figure S2. Correlation of Tax and HSP70 expression: The HTLV-I-infected cell lines, ATL-026i and ATL-056i, were either mock treated (37 °C) or exposed to heat stress at 43 °C for 30 min. Tax and HSP70 expression one day after HS are shown in dot plot.

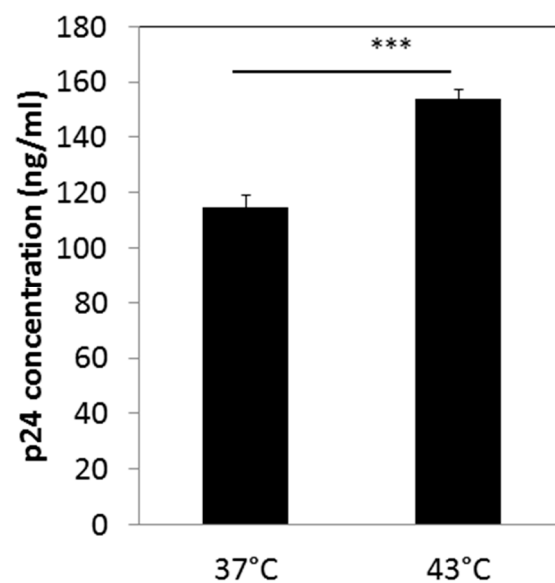


Figure S3. HS increased p24 concentration: The HTLV-I-infected cell lines, YT/cM1 was either mock treated or heat shocked. Concentration of p24 in culture supernatant was measured by ELISA at two days after HS. The values denote the means \pm SD. *** $p < 0.001$.

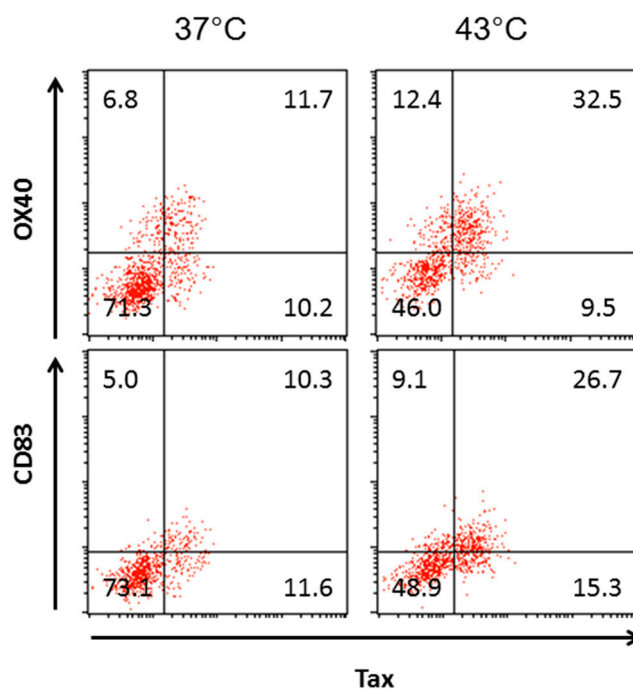


Figure S4. Correlation between intra-cellular Tax and cell surface expression of OX40 and CD83: The HTLV-I-infected cell line, ATL-026i, was mock treated (37 °C) or heat shocked at 43 °C or for 30 min. After 24 h cultivation, cells were analyzed for simultaneous expression of Tax, OX40 and CD83.

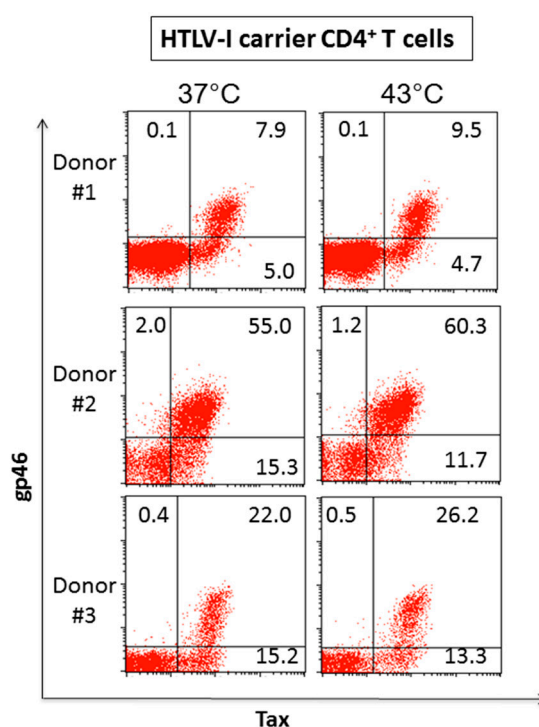


Figure S5. HS upregulated expression of Tax and gp46 in fresh PBMC from carriers: Aliquots of diluted whole blood from three different HTLV-I carriers were either exposed to heat shock (43 °C) or mock treated, and then cultured for 24 h. The gated population of CD4⁺ T cells was analyzed for the expression of Tax and gp46 antigens by FCM.

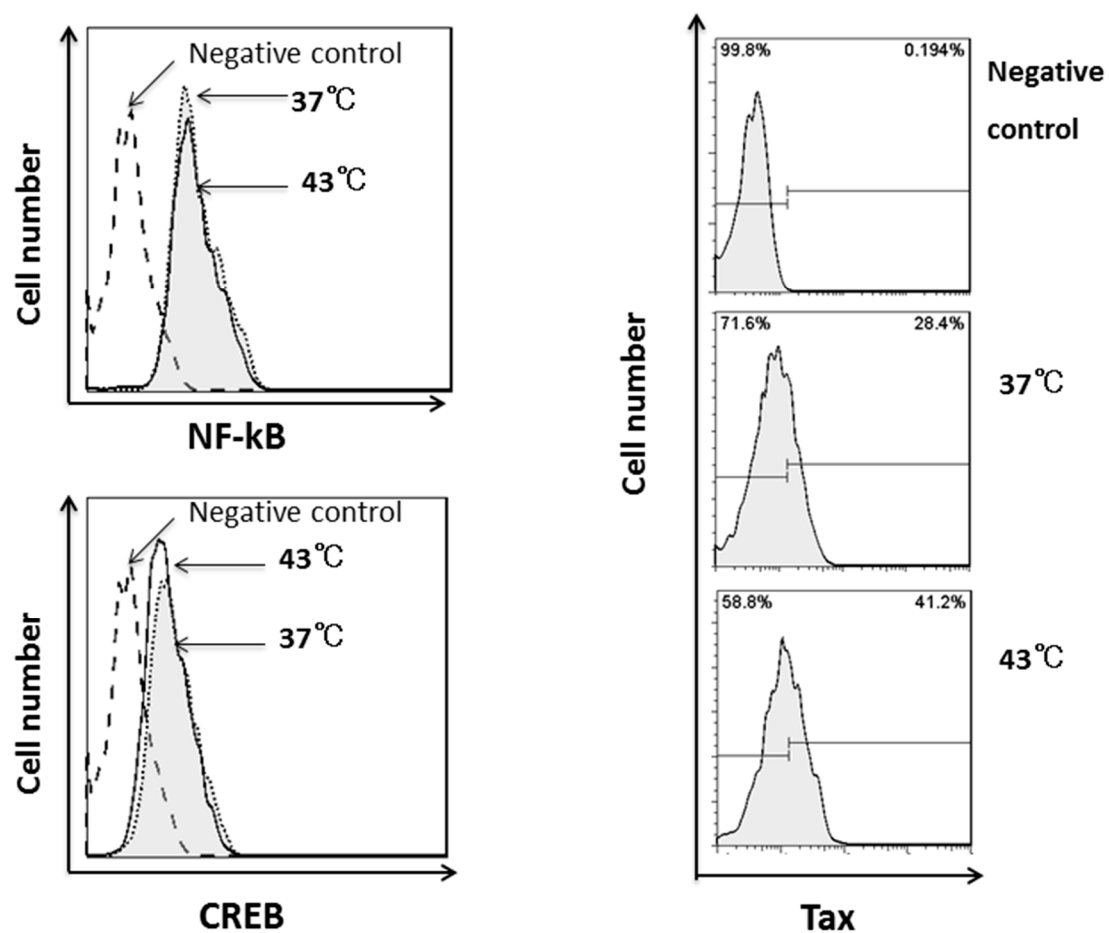


Figure S6. Effect of HS on the expression of NF-kB and CREB molecules: HTLV-I-infected cell line ATL-056i was mock treated (37 °C) or heat shocked. After 24 h incubation, the intracellular expression of NF-kB and CREB was analyzed by FCM (left panel). Primary antibodies used were rabbit mAbs anti-NF-kB p65 antibody (E379) and anti-CREB antibody (E306) purchased from Abcam (Cambridge, MA, USA) followed by staining with donkey anti-rabbit IgG-FITC (Biolegend, SanDiego, CA, USA). Negative control showed staining with only 2nd anti-rabbit IgG antibody. Tax expression of these cells is shown in right panel.

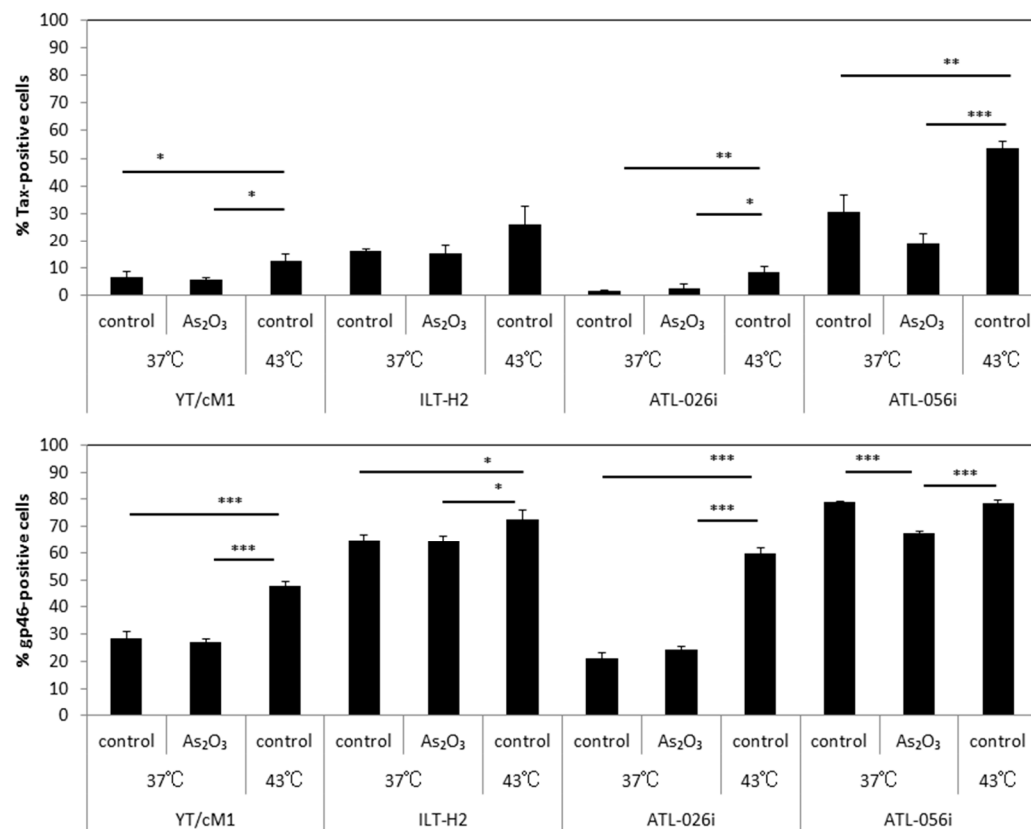


Figure S7. The failure of exposure to oxidative stress, As₂O₃, to induce increased expression of Tax and gp46: The HTLV-I-infected cell lines YT/cM1, ILT-H2, ATL-026i and ATL-056i, were either mock treated or exposed to heat stress at 43 °C for 30 min or oxidative stress in the presence of 0.25~1.0 μM As₂O₃, and then cultured for 24 h. The frequencies of Tax and gp46 expression were analyzed by FCM. The values denote the means ± SD. **p*<0.05, ***p*<0.01, ****p*<0.001.

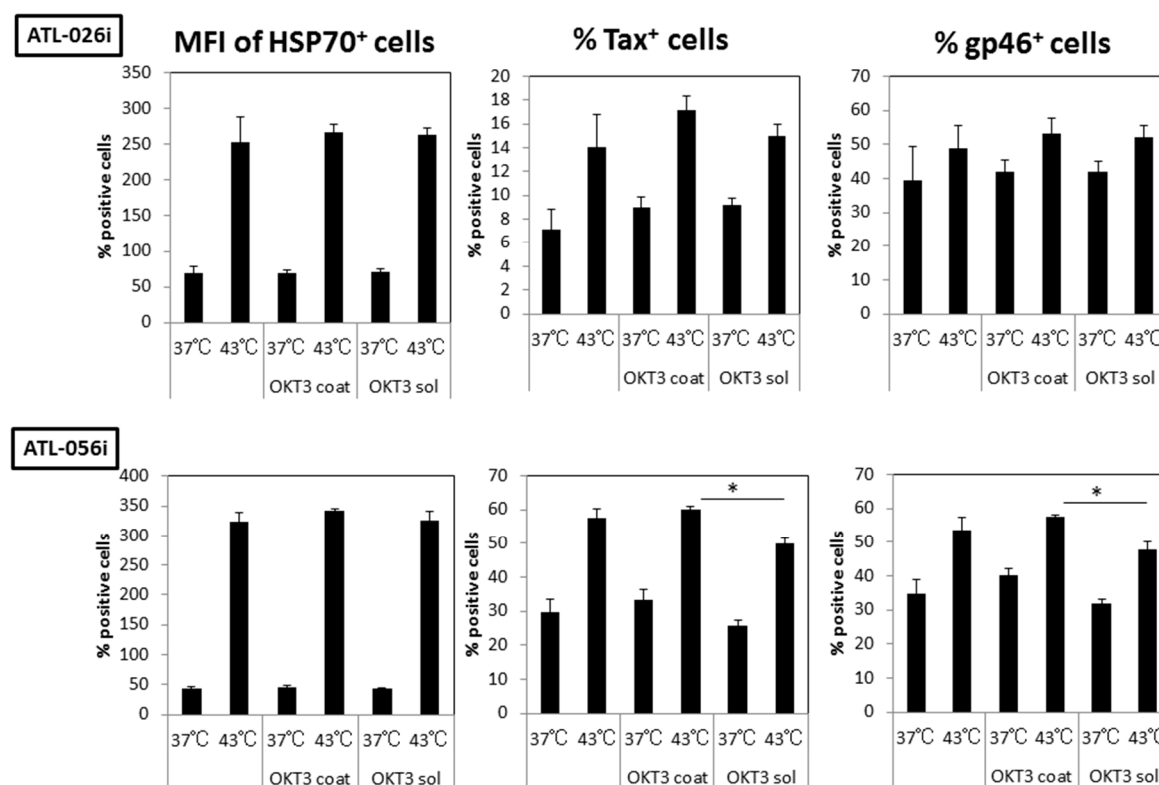


Figure S8. Failure to stimulate HTLV-I-infected cell lines with anti-CD3 mAb (OKT-3): ATL patient-derived HTLV-I-infected cell lines, ATL-026i and ATL-056i, were mock treated (37 °C) or exposed to heat stress, cultured alone or in the presence of either immobilized OKT-3 (OKT-3 coat) or soluble OKT-3 (2 µg/mL) (OKT-3 sol) for 24 h. Then, Tax, gp46 and HSP70 expression were analyzed by FCM.

