**Supplemental material and methods**

### Human cells and culture conditions

CD4+ T cells were sorted by MACS magnetic negative selection (Miltenyi Biotec, Bergish Gladbach, Germany) according to manufacturer’s instructions (purity >95% assessed by FACS) and cultured (2x106) in 2 ml of complete medium (RPMI-Gibco, Life Technologies, Carlsbad, CA, USA-containing 10% FCS) in the absence or presence of the Tat protein in 24-well flat bottomed polystyrene plates pre-coated overnight at 4 °C with PBS or anti-CD3 mAb (0.5 µg/ml; R&D Systems, MN, USA). After cells seeding, soluble anti-CD28 mAb (0.1 µg/ml; R&D Systems) and Tat protein were added where indicated.

Naïve CD4+ T cells were sorted by MACS magnetic selection (Naive CD4+T Cell isolation kit II, Miltenyi Biotec), according to manufacturer’s instructions (purity >95% assessed by FACS) and cultured in non-polarizing condition. Briefly, Naïve CD4+ T cells were activated with anti-CD3/CD28 in the presence of Transforming Grow Factor 1 (TGFβ-1, 1 µg/ml; Miltenyi Biotec), anti-IL12 (2 ng/ml; Miltenyi Biotec) and anti-IL4 (10 ng/m; Miltenyi Biotec) mAbs. Medium was changed after every 3 days with fresh complete medium containing IL2 (100 ng/ml) and, where indicated, Tat (0.1 μg/ml).

**Surface staining**

All stainings were carried out in FACS buffer (PBS + 1% FCS) for 30 min at 4 °C with the following monoclonal antibodies: HLA-DR FITC, CD38 PE/Dy 747, CD25 FITC, CD4 PE, CD27 PE (ImmunoTools, Friesoythe, Germany); CD4 Qdot 705 (Life Technologies, Monza, Italy); CD45RA PerCP (Miltenyi Biotec); CCR7 FITC (BD Biosciences, San Jose, CA, USA). Data were acquired on a BD FACScan and analyzed using BD Cell Quest Pro software.

**Intracellular Staining (ICS)**

For characterization of Tat-specific T cell responses, an 8-colour ICS assay was performed on PBLs as previously detailed (Bauer A et al 2017, in press). Briefly, PBLs were stimulated for 18 hours in 96-well flat bottomed polystyrene plates coated overnight at 4 °C with PBS and anti-CD3 mAb (1.5 µg/ml). Soluble anti-CD28 mAb (1 µg/ml, BD), anti-CD49d (1 µg/ml BD), protein transport inhibitor containing monensin/BD Golgistop (5 μg/ml, BD), Brefeldin A (5 ug/ml, Sigma-Aldrich, Munich, Germany), CD107a-FITC (eBioscience, San Diego, CA, USA) and, when required, Tat, were added after cells seeding (1 x 106 cells per well in 0.2 ml of complete medium). After stimulation, PBLs were washed once with PBS and incubated with 20 mM EDTA for 15 minutes at room temperature. Subsequently, surface proteins (CD4 and CD8) were stained for 20 minutes and cells were washed twice with FACS buffer (PBS, 1% FCS). The cells were permeabilized using the Cytofix/Cytoperm kit (Becton Dickinson, BD, San Diego, CA, USA) and stained with anti-CD3 APC-Cy7, anti-IFNγ V450, anti-IL-2 APC and anti-TNFα PE Cy7 antibodies (BD). Cells were then washed twice, fixed in CellFix (BD) and analyzed with a FACScanto II. Electronic compensation was conducted with antibody capture beads (BD) stained separately with the individual antibodies used in the test samples. Flow cytometry data were analyzed using FlowJo (version 8.8.3; Tree Star, Inc.).

**Reverse transcription (RT) and quantitative real time PCR**

DNase-treated total RNA was isolated from cells using Trizol reagent (Life Technologies) according to the manufacturer's instructions and used to perform cDNA synthesis (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Life Technologies). cDNA was PCR-amplified with a Chromo4 real-time PCR Detection System using Kapa SYBR Green Fast qPCR Kit (Kapabiosystems, Wilmington, MA, USA) according to the manufacturer's recommendations with the following cycle conditions: 3 minutes at 95°C, then 40 cycles of 15 seconds at 95°C, and 20 seconds at 60°C. Quantitative PCR was performed using the following pairs of primers (TEMA ricerca, Bologna, Italy):

IL2 forward 5’-AAGAATCCCAAACTCACCAGG-3’,

IL2 reverse 5’-ATTGCTGATTAAGTCCCTGGG-3’,

IFNγ forward 5’-TGACCAGAGCATCCAAAAGAG-3’,

IFNγ reverse 5’-CGACAGTTCAGCCATCACTTG-3’,

TNFα forward 5’-GAACCCCGAGTGACAAGC-3’,

TNFα reverse 5’-TGGGAGTAGATGAGGTACAGG-3’,

T-bet forward 5’-GCGCCAGGAAGTTTCATTTG-3’,

T-bet reverse 5’-GGAAAGTAAAGATATGCGTGTTGG-3’,

Eomes forward 5’-TCATTACGAAACAGGGCAGG-3’,

Eomes reverse 5’-TGCATGTTATTGTCGGCTTTG-3’.

The relative amount of each RNA was calculated by the 2−ΔΔCT method using human 18S as housekeeping gene (18S forward 5’-GTAACCCGTTGAACCCCATT-3’ and 18S reverse 5’-CCATCCAATCGGTAGTAGCG-3’.). CT values are the mean of two biological replicates and each assay was performed at least twice.

**Fig. S1. Effect of different Tat-concentrations and inhibition by anti-Tat antibodies.** CD4+ T cells purified from healthy donors (n=3) activated with anti-CD3/CD28 were cultured in the absence or presence of soluble Tat at concentrations ranging from 0.001 to 1 µg/ml (a). After 4 hours, IL-2, IFNã, TNFá mRNA levels were quantified by qPCR and normalized to untreated cells. CD4+ T cells purified from healthy donors (n=3) activated with anti-CD3/CD28 were cultured in the absence or presence of 0.1 µg/ml of soluble Tat +/- anti-Tat immune or mock sera (b). After 4 hours, IL-2, IFNã, TNFá mRNA levels were quantified by qPCR and normalized to untreated cells.



**Fig. S2. Tat does not modulate the phenotype of CD4+ T cells.** PBLs from healthy donors unstimulated or activated with anti-CD3/CD28 were cultured in the absence or presence of Tat (0.1 µg/ml). The percentage of CD4+ T cells expressing CD69, CD25, CD38 and HLADR was measured after 10 (CD69) and 24 (CD25, CD38 and HLADR) hours by flow cytometry. Mean +/- SEM of data normalized to untreated cells (NT) are shown (n=5).

