**Supplemental Figure 1. HIV-1/VSV Infection of BMM. (A)** Representative histograms showed the purity of BMM determined by CD11b expression after 7 days differentiation. **(B)** Representative histograms showed the expression of HIV-1p24 in BMM infected by HIV-1/VSV at 1 pg of HIV-1p24/cell for 24 hours. **(C)** Immunohistochemistry staining plus light microscopy showed the level of HIV-1p24 staining increased in parallel to the virus infective dose. White scale bars represent 100 μm.

**Supplemental Figure 2. Murine Tregs Expanded by T-Activator CD3/CD28 Maintain Foxp3 Expression and Suppressive Activities. (A)** The purity of isolated Treg was determined by expression of $CD4^{+}CD25^{hi}$ using flow cytometric analysis (Representative dot plots are shown from five independent experiments). **(B)** The purity of isolated Treg was determined by expression of $CD25^{hi}Foxp3^{+}$ using flow cytometric analysis (Representative dot plots are shown from five independent experiments). **(C)** The expression of Foxp3 in expanded Treg was determined by flow cytometric analysis (Representative histogram are shown from five independent experiments). **(D)** Graphs represent the mean percentage of expressions of CD25 and Foxp3 in Tcon and Treg before and after expansion (data were pooled from five independent experiments). Each bar represents a mean ± SD. **(E)** The suppressive capacity of expanded Treg was evaluated by CFSE labeled Tcon proliferation suppression assay (The numbers in blue squares show the percentage of $CFSE^{low}$ cells, which indicates the percentage of divided cells). **(F)** The capacity of Tcon proliferation suppression by expanded Treg increased with higher Treg/Tcon ratio. Each bar represents a mean ± SD. ***$p < 0.001$.

**Supplemental Figure 3. Expanded Human Tregs Maintain Their Suppressive Activities. (A)** The ratios of CD4/CD8 T cells and purity of isolated Treg were determined by expression of $CD4^{+}CD25^{hi}$ using flow cytometric analysis (a: before CD4 T cell enrichment, b: after CD4 T cell enrichment, c: before CD25-Microbeads positive selection, d: after passing the $1^{st}$ column, e: after passing the $2^{nd}$ column). **(B)** The suppressive capacity of expanded Treg or Tcon was evaluated by CFSE labeled Tcon proliferation suppression assay (The numbers in blue squares show the percentage of $CFSE^{low}$ cells, which indicate the percentage of divided cells).

**Supplemental Figure 4. Human Treg Induced HIV-$1_{ADA}$ Infected Human MDM Apoptosis Through Mitochondrial Pathways. (A)** Activation of Caspase-1 (upper panel), Caspase-8 (middle panel) and Caspase-9 (bottom panel) was measured by FLICA assay plus immunofluorescence microscopy. Treg treated HIV-$1_{ADA}$ infected MDM showed decreased Caspase-1 activation and increased Caspase-9 activation, which indicates that such cell death is apoptosis through mitochondrial pathways. White scale bars represent 100 μm. **(B)** Confocal immunofluorescence of HIV-$1_{ADA}$ infected MDM with specific monoclonal antibodies against Apo2.7 show brighter staining of Apo2.7 (red) in HIV-$1_{ADA}$ infected MDM cocultured with Treg. Data shown are representative of three independent experiments. White scale bars represent 100 μm. **(C)** Treg induces HIV-$1_{ADA}$ infected MDM giant cell shrinkage as visualized by morphology and HIV-

1p24 immunohistochemistry. Data shown are representative of three independent experiments. White scale bars represent 100 μm.

**Supplemental Figure 5. Treg Express Granzyme A, B and Perforin. (A)** Confocal immunofluorescences of activated murine Tcon (middle column) and Treg (right column) with specific monoclonal antibodies against granzyme A (upper panel), granzyme B (middle panel) and perforin (bottom panel) show cytoplasmic granular staining of granzyme A (red), B (red) and perforin (red). Treg showed larger size and more abundant granules than Tcon. Data shown are representative of five independent experiments. White scale bars represent 5 μm. **(B)** Representative histograms showed the expression of granzyme A, granzyme B and perforin by activated murine Tcon and Treg measured using intracellular immunofluorescence staining plus flow cytometry. Treg express higher intensity of granzyme A, granzyme B and perforin than Tcon. Dashed line was drawn according to isotype control. **(C)** Representative histograms showed the expression of granzyme A, granzyme B and perforin by activated human Tcon and Treg measured using intracellular immunofluorescence staining plus flow cytometry. Treg express higher intensity of granzyme A, granzyme B and perforin than Tcon.

**Supplemental Figure 6. Treg Induced Cytotoxicity of Infected BMM is Cell Contact Dependent. (A)** Light microscopic evaluation of BMM mitochondrial activities by Tcon or Treg cocultivation, respectively. MTT stain is illustrated. (**B)** Change in MTT activity and cell viability of HIV-1/VSV-infected BMM cocultured with Tcon or Treg. The

cytotoxicity of Treg on infected BMM is dependent on cell-cell contact (Values shown as a percentage of control). White scale bar represents 50 μm. Each bar represents a mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

**Supplemental Figure 7. Mechanisms for Treg Immunoregulation of HIV-1 Infected Macrophages.** Treg functions include functional transformation, apoptosis induction, and antivirus immunity, which may substantively affect the onset and tempo of HAND neurodegeneration.

**Supplementary Table 1**. **Concentration of Cytokines in BMM Conditioned Media**. *a* = Cytokines were detected by cytometric bead array. *b* = HIV-1p24 were detected by ELISA.

**Supplementary Table 2. Proteins Identified Following Filtering of iTRAQ Data.** Proteins were identified from the mixtures of iTRAQ labeled peptides according to the filtering procedures described in the Materials and Methods section. Ratios of tagged proteins are listed for those associated with BMM viral infection (115) compared to uninfected BMM (114) [114:115] and infected BMM with Treg (117) or with Tcon (116) compared to infected BMM alone [117:115 and 116:115 respectively].