

**Supplementary Figures for:  
Modulation of Interleukin-12 activity in the presence of heparin**

Srinivas Jayanthi<sup>1#</sup>, Bhanu prasanth Koppolu<sup>2,3#</sup>, Khue G. Nguyen<sup>4,5#</sup>, Sean G. Smith<sup>2,3</sup>, Barbara K. Felber<sup>6</sup>, Thallapuram Krishnaswamy Suresh Kumar<sup>1,4\*</sup>, David A. Zaharoff<sup>2,3,4,5\*</sup>

<sup>1</sup> Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR

<sup>2</sup> Department of Biomedical Engineering, University of Arkansas, Fayetteville, AR

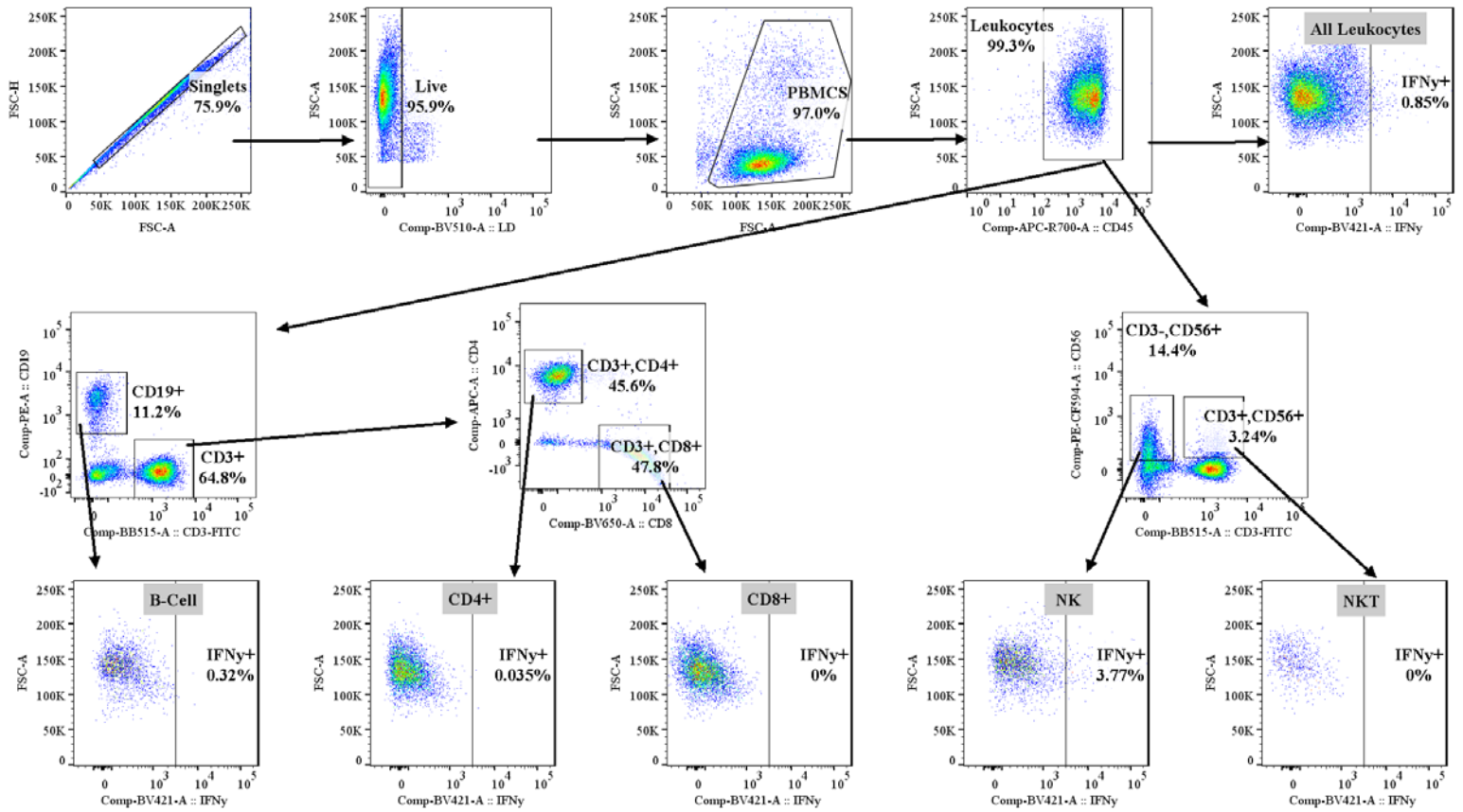
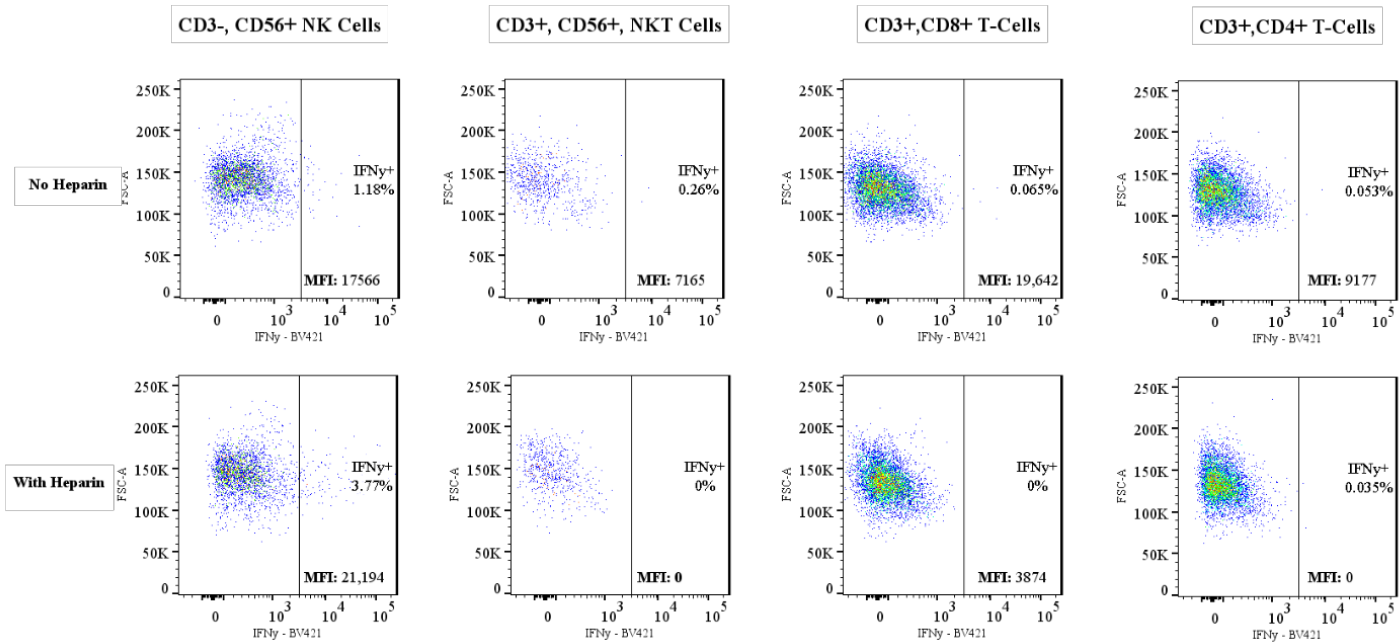
<sup>3</sup> Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina-Chapel Hill, Raleigh, NC

<sup>4</sup> Cell and Molecular Biology Program, University of Arkansas, Fayetteville, AR

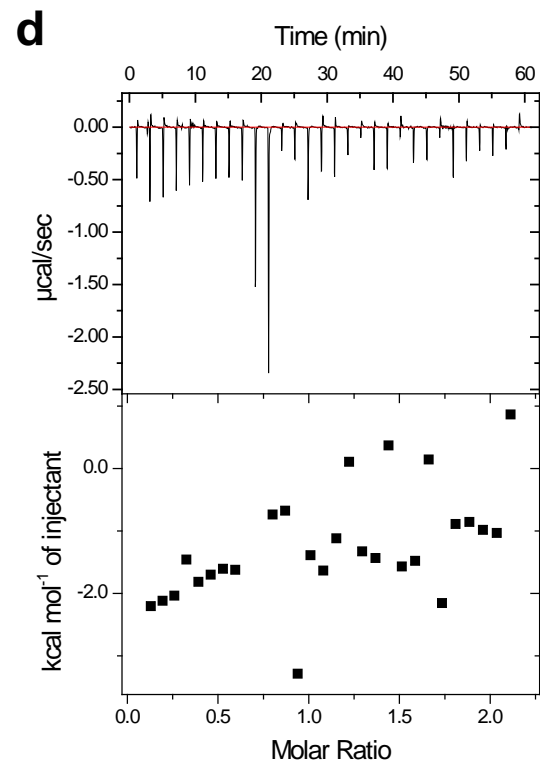
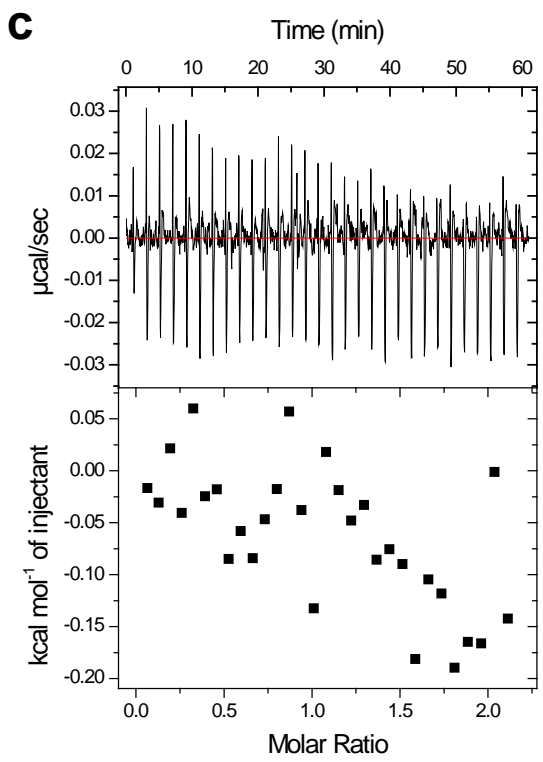
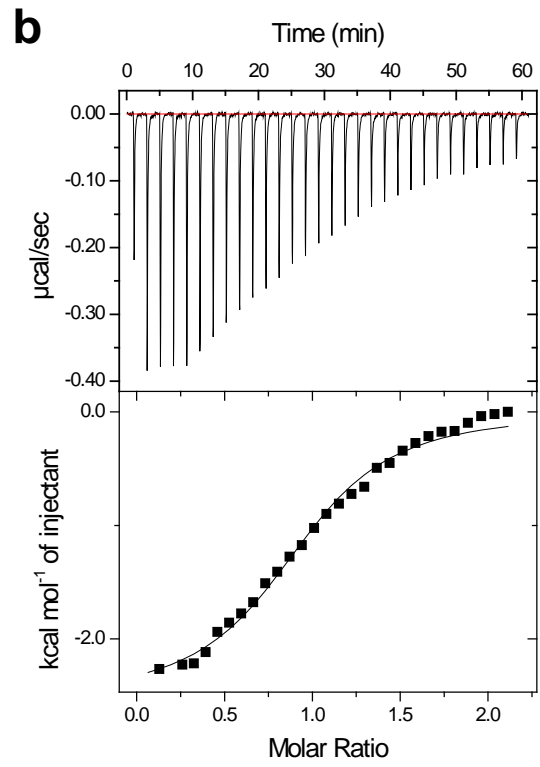
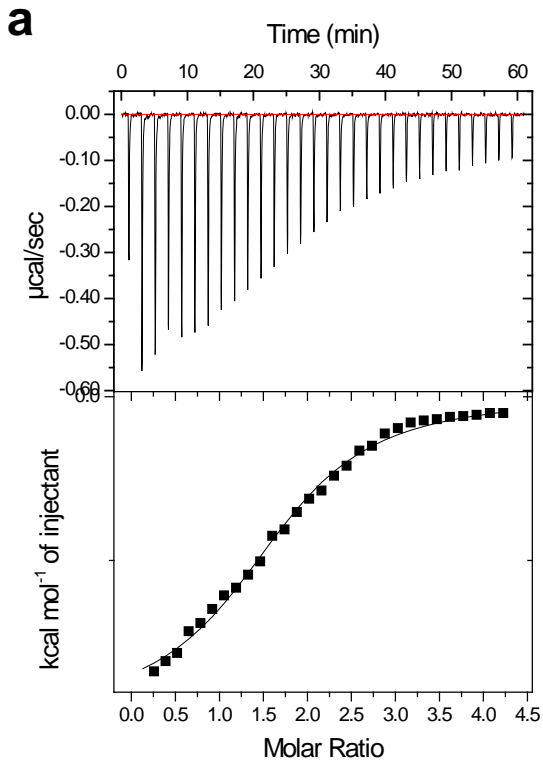
<sup>5</sup> Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC

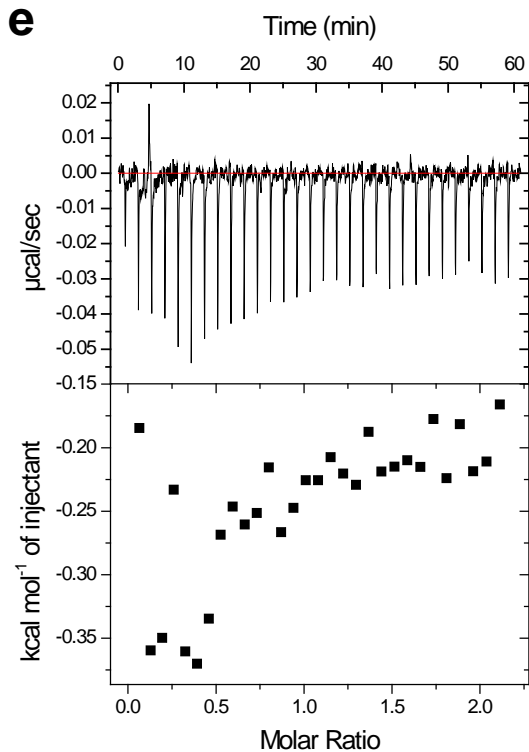
<sup>6</sup> Human Retrovirus Pathogenesis Section, Vaccine Branch-National Cancer Institute, Frederick, MD

# Authors contributed equally

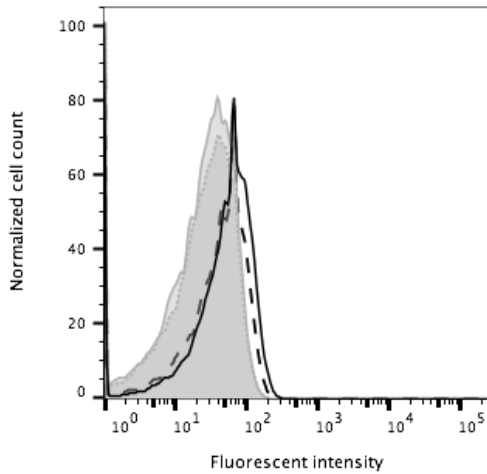
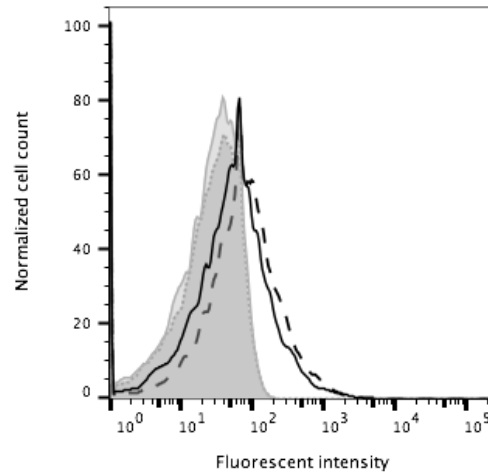
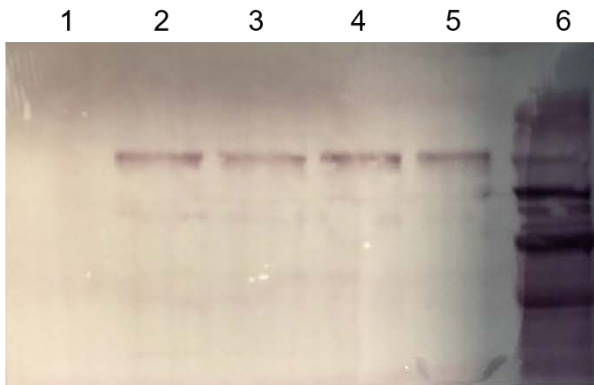
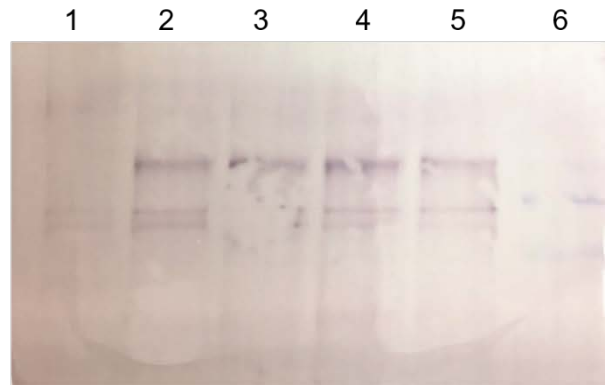
**a****b**

**Fig. S1. Intracellular IFN- $\gamma$  expression among PBMC subsets.** Freshly isolated human PBMCs were stimulated with hIL-12  $\pm$  heparin and analyzed as described in Supplementary Methods. (a) Gating strategy for analysis of intracellular IFN- $\gamma$  expression. IFN- $\gamma$  positive populations were determined based on background production in cells that were not stimulated with IL-12. (b) PBMC subset analysis revealed that natural killer cells are the primary responders to hIL-12+heparin stimulation. NK: natural killer cells; NKT: natural killer T-cells; PBMCs: peripheral blood mononuclear cells; IL-12: Interleukin-12; IFN $\gamma$ : Interferon-gamma.





**Figure S2. Isothermograms for hIL-12 interactions with various GAGs.** Isothermograms describe binding interactions between hIL-12 and (A) heparin, (B) heparin sulfate, (C) chondroitin sulfate, (D) hyaluronic acid, or (E) dextran. The upper panel of each isothermogram shows the raw data obtained for each of the 30 injections. The lower panels display the best fit data to one-set of sites binding model using Origin<sup>TM</sup> v7.0 software.

**a****b****c****d**

**Figure S3. Analysis of IL12R expression in mutant and wild-type NK92MI cells.** IL12R $\beta$ 1<sup>mut</sup>/IL12R $\beta$ 2<sup>mut</sup> and wild-type NK-91MI were stained with antibodies against IL12R $\beta$ 1 and IL12R $\beta$ 2 and analyzed via flow cytometry as indicated in Supplementary Methods. (A) IL12R $\beta$ 1 expression in wild-type NK-91MI cells (solid line) and IL12R $\beta$ 1<sup>mut</sup>/IL12R $\beta$ 2<sup>mut</sup> NK-92MI cells (dashed line). (B) IL12R $\beta$ 2 expression in wild-type NK-91MI cells (solid line) and IL12R $\beta$ 1<sup>mut</sup>/IL12R $\beta$ 2<sup>mut</sup> NK-92MI cells (dashed line). Isotype controls for wild-type NK-92MI cells (filled-solid line) and IL12R $\beta$ 1<sup>mut</sup>/IL12R $\beta$ 2<sup>mut</sup> NK-92MI cells (filled-dashed line) are displayed in each panel. Data are representative of three independent experiments. Western blots for IL12R $\beta$ 1 (C) and IL12R $\beta$ 2 (D) were developed as described in Supplementary Methods. *Lane 1*: HEK-293 cells; *Lanes 2-4*: IL12R $\beta$ 1<sup>mut</sup>/IL12R $\beta$ 2<sup>mut</sup> NK-92MI cells; *Lane 5*: wild-type NK-92MI; *Lane 6*: protein ladder.